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Bioassay with quantal response observed at different times

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BIOASSAY WITH QUANTAL RESPONSE OBSERVED AT DIFFERENT TIMES

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I. INTRODUCTION

A. Quantal Response Bioassay

Suppose that random samples of experimental subjects are exposed to various doses of some stimulus to each of which a subject may or may not respond. This is a particular form of bioassay in which the response is quantal. A dose is said to be effective for a subject if it produces the required response in the subject and the minimum effective dose for a subject is called its tolerance (17). One of the main objects of quantal bioassay is to estimate the mean tolerance.

Clearly a situation might arise where in place of a simple dichotomy, subjects may respond in more than two ways to any dose of the stimulus. Accordingly we envisage an experiment where random samples of experimental subjects are exposed to varying dose levels of a stimulus and, as a result of the application of the dose, each subject can be placed in one of more than two mutually exclusive and exhaustive classes. A straightforward illustration of such an experiment in which insects subjected to a poison were classified as 'alive', 'moribund', and 'dead' is given by Dahm and Gurland (12).

For many treatments (drugs, vitamins, for example) there is a finite interval between the application of a stimulus

and the corresponding reaction. To be specific, suppose that a dose z is administered to a batch of experimental subjects which are examined at a number of specified times t, then the dose-response relation can be written generally as follows:

$$p = f(z, t) \tag{1}$$

where p is the proportion observed to be affected.
The equation in (1) may be rewritten as follows:

$$F(p, z, t) = 0, \tag{2}$$

which, since p is a proportion, represents a surface having asymptotes defined by the planes p = 0 and p = 1. In general it may be said that the analysis of quantal response bioassay usually amounts to the estimation of some form of the functional relationship given in (2).

B. Statement of the Present Problem

Suppose that doses $z_1, z_2, \ldots, z_m$ are administered to numbers $n_1, n_2, \ldots, n_m$ subjects respectively and that the times of observation $t_1 \quad t_2 \quad \ldots \quad t_k$ are pre-specified for all dose levels. At the end of each one of these time intervals every individual subject is examined and classified, according to its response, into one of the mutually exclusive classes 'dead', 'moribund', and 'alive'. At the conclusion

of an experiment, there will be for each subject, an individual history of its survival time, the time for which it stayed in the state of being 'moribund', and the time at which it was observed as 'dead'.

The main problem considered in this study is the introduction of a suitable procedure for estimating parameters and ED_{50} values by utilizing all information on each individual subject. This procedure is here developed by using a simple birth process to formulate a mathematical model which appears to be appropriate to this particular situation.

In the first six chapters, unless specifically stated, it is assumed that no experimental subjects once observed as 'moribund' recover sufficiently to become 'alive'. A procedure for estimating parameters when such recovery is possible is discussed in the Appendix. For simplicity the trichotomous quantal response is primarily considered, the principles, however, can be readily extended to the case of polychotomous quantal response.

II. REVIEW OF THE LITERATURE

A. Response-Time Distribution and Trichotomous Quantal Response

Sampford (26), (27), (28) has studied dichotomous quantal response-time distributions to estimate a relationship which may be used to predict the time required for a given proportion of individuals to respond to a single dose, correspondingly the proportionate response to be expected at a given time can also be obtained. Sampford's method is based on the assumption that the response-time metameter is normally distributed.

White and Graca (30) introduced a method for analyzing dichotomous quantal response data in which the responses have been grouped into intervals by the fact that observations are made only at several pre-specified times. In principle this amounts to an extension of Sampford's procedure to the case where more than one dose is used. Since the numbers of the responses are accumulated, the responses at successive times are not independent. White and Graca partly resolved this difficulty by estimating parameters using a minimum modified chi-square method on the successive differences between the numbers observed at the various times.

Recently, Gurland, et al. (19) used a minimum chi-square procedure for the analysis of polychotomous quantal response

data. In particular, these authors demonstrated that when biological responses are polychotomous, it is more efficient to use this information explicitly in analyzing the data rather than to pool certain outcomes to obtain the dichotomous response situation.

B. Application of Simple Stochastic Processes to Biology

Since Kolmogorov's famous paper of 1931 'On Analytical Methods in the Theory of Probability', the theory of stochastic processes has been rapidly developed and it has been shown that the theory can be successfully applied to many practical problems.

During the past decade, a problem that has received much attention is the estimation of intensities of mortality, recovery, and relapse in follow-up studies. Some examples of work in this area are:

(a) Application of the theory of homogeneous Markov processes to follow-up studies of cancer patients, Fix and Neyman (18).

(b) The use of a simple birth process in follow-up studies, Littell (23).

(c) General problems which arise in application of the theory of stochastic processes to follow-up studies are discussed by Zahl (31).

(d) A study by Chiang (11) on the general stochastic model of population growth in experimental studies on flour beetles.

From an examination of the literature it appears, however, that very little research has, so far, been carried out on the direct application of theory of stochastic processes to bioassay analysis.

III. IDENTIFIABLE AND UNIDENTIFIABLE SUBJECTS IN BIOASSAY EXPERIMENTS

A. Preliminary Remarks

Quantal response bioassay experiments in which individual subjects are observed as different times can be classified as either,

(a) experiments in which individual subjects are not identifiable, or

(b) experiments in which individual subjects are identifiable.

Bioassay experiments in which insects, such as house flies or fruit flies, are used as subjects usually belong to the first category. The second category includes bioassay experiments in which animals, such as mice or rabbits are employed as experimental subjects.

B. Dichotomous Quantal Response Bioassay

In the case of dichotomous quantal response bioassay experiments in which observations are made after each of a number of time intervals, the subjects need not be identifiable since the difference between the numbers of individuals observed as 'dead' at time j and those observed as 'dead' at time (j-1) will be due only to individuals which were in the single class 'alive' at time (j-1).

Accordingly the original n subjects exposed to dose i can, at the end of the j-th time interval, be classified according to the following set of exhaustive, independent, and mutually exclusive categories.

(a) 'dead' at the end of time (j-1) and 'dead' at the end of time j.

(b) 'alive' at the end of time (j-1) and 'dead' at the end of time j.

(c) 'alive' at the end of time (j-1) and 'alive' at the end of time j.

The probabilities that randomly selected subjects will be found in one of these three classes can be readily estimated from the observed numbers of individual subjects in the corresponding classes and this was, in fact, the principle underlying the method of the analysis described in (26), (30).

C. Trichotomous Quantal Response Bioassay

1. Individual subjects not identifiable

For the trichotomous case with observations on the numbers of subjects which were 'dead', 'moribund', and 'alive' at the end of the j-th time interval, the corresponding set of mutually exclusive, exhaustive, and independent classes is:

(a) 'dead' at the end of time j and 'dead' at the end of time (j-1)

(b) 'dead' at the end of time j and 'moribund' at the end of time (j-1)

(c) 'dead' at the end of time j and 'alive' at the end of time (j-1)

(d) 'moribund' at the end of time j and 'moribund' at the end of time (j-1)

(e) 'moribund' at the end of time j and 'alive' at the end of time (j-1)

(f) 'alive' at the end of time j and 'alive' at the end of time (j-1).

Here, as distinct from the dichotomous case, it can be seen that when the individual subjects are not identifiable the numbers of individuals in classes (b), (c), (d), and (e) are not observable. It follows that we cannot estimate all of the appropriate probabilities corresponding to each of the six classes. Accordingly, when individual subjects are not identifiable, a technique of estimation for the trichotomous case analogous to the modified minimum chi-square method employed by White and Graca for the dichotomous case is not now available.

The required parameters can, however, be easily estimated if the class 'moribund' is pooled with the class 'dead' or with the class 'alive'. This essentially amounts to the analysis of dichotomous quantal response bioassay and an improved estimate might be obtained by combining estimates,

one obtained from the frequencies of 'dead' subjects only and the other obtained when the classes 'dead' and 'moribund' are pooled.

2. Individual subjects identifiable

The preceding procedure can also be applied to the case where the individual subjects responding in the ordered, mutually exclusive and exhaustive classes after various times can be identified. The alternative methods, which are considered to be superior in this case, constitute the subject of the present study.

IV. USE OF A SIMPLE STOCHASTIC MODEL IN TRICHOTOMOUS QUANTAL RESPONSE

A. Preliminary Remarks

We are interested in the changes with time in the biological responses of individual subjects exposed to a certain toxicant. We accordingly consider random variates which can assume one of three specific values (the response classes in this case) at every moment over some finite time interval. A process giving rise to observations that is, to particular values of the variates, in such a context is said to be stochastic. Let us define the possible response classes 'dead', 'moribund', and 'alive' as possible 'states'. Then a random variate x which can be classified into one and only one such state at time t, $(0 \leq t \leq T)$, is stochastic. More formal definitions of a stochastic process, Markov chain, Markov process and other terms which frequently appear in the study of stochastic processes are given in, for example, (9), (13), and (29).

The event that an individual subject is transferred from one state to another between two successive times of observation will be called 'transition'. It is assumed that such transition is governed by a set of probabilistic laws.

The purposes of this chapter are:

(a) to formulate a mathematical model which appears to be relevant in this particular situation.

(b) to derive the probability functions which characterize the transitions of the individual subjects over a finite real time interval.

For the purpose of simplifying the presentation, the case of trichotomous quantal responses is discussed in this chapter. The extension to any number of response classes is, however, possible without any extension in principle.

B. The Model and its Qualifying Assumptions

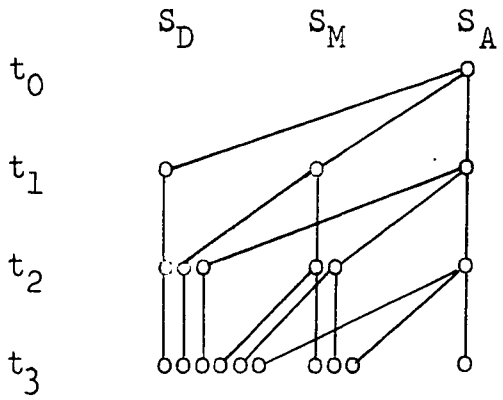
Let us denote the three possible states in which an individual may be observed at a given time as:

(a) S_A : The state of being 'alive'.

(b) S_M : The state of being 'moribund'.

(c) S_D : The state of being 'dead'.

Here S_D is the terminal state. A scheme representing the three states and the possibilities of passage from one state to another is given in Figure 1 below for the first three successive time intervals.



(Figure 1)

1. Biological assumptions

(a) Throughout the present chapter it will be assumed that the transfer from S_M to S_A does not occur at any time during the total period of the experiment. In many experiments, however, this assumption will not be strictly true and situations where the restriction on recovery is relaxed will be discussed in the Appendix.

(b) In the present study it is assumed that the total experimental time is so short that the rates of moribundity and mortality due to causes other than the toxicant (natural and accidental death, for example) can be neglected.

2. Mathematical assumptions

(a) If an individual is in state S_A at time s, for example, we assume that there exist conditional probabilities that at a later time t, $(s<t)$, the individual will be found in one of the states S_A, S_M, or S_D. Thus, for any two given times s and t, $0 \leqslant s < t \leqslant T$, where T is the total period of observation, we postulate the existence of the following conditional probabilities.

$$P_{AA}(s, t), \quad P_{AM}(s, t), \quad P_{AD}(s, t), \quad P_{MM}(s, t), \quad P_{MD}(s, t)$$

where $P_{AM}(s, t)$, for example, is the conditional probability that a random individual which is in state S_A at time s will be found in state S_M at time t. These transition

probabilities may be represented in matrix forms:

$$\begin{bmatrix} P_{AA}(s, t) & P_{AM}(s, t) & P_{AD}(s, t) \\ 0 & P_{MM}(s, t) & P_{MD}(s, t) \\ 0 & 0 & 1 \end{bmatrix}$$

in which

$$P_{AA}(s, t) + P_{AM}(s, t) + P_{AD}(s, t) = 1$$

and

$$P_{MM}(s, t) + P_{MD}(s, t) = 1 \qquad (3)$$

(b) It is assumed that the present model represents a homogeneous, stationary Markov process. The following conditions are therefore required.

(i) The transition probabilities given in (3) are independent of the history of the individual up to its observation at time s.

(ii) The transition probabilities depend only on the interval (t-s) and the pair of states at times s and t, and are independent of s, or equivalently, the probability distribution of the Markov process of the present study is invariant under the time translation.

(c) The additional assumptions that characterize the model under discussion are as follows:

$$\text{(i)} \quad \lim_{t \to s} P_{AA}(s, t) = \lim_{t \to s} P_{MM}(s, t) = 1$$

$$\text{(ii)} \quad \lim_{t \to s} P_{AM}(s, t) = \lim_{t \to s} P_{AD}(s, t)$$

$$= \lim_{t \to s} P_{MD}(s, t) = 0$$

(iii) The transition probabilities given in (3) have partial derivatives at $t = s$. (4)

(d) In consequence of the assumptions given in (4), the following regularity conditions which specify the homogeneous Markov process under the present study can now be deduced as in (9) and (15).

(i) To states S_A and S_M there correspond transition intensities $\xi_{AM} \geq 0$ and $\xi_{MD} \geq 0$ such that,

$$\lim_{\Delta t \to 0} \frac{1 - P_{AA}(t, t+\Delta t)}{\Delta t} = \xi_{AM} \tag{5}$$

$$\lim_{\Delta t \to 0} \frac{1 - P_{MM}(t, t+\Delta t)}{\Delta t} = \xi_{MD} \tag{5a}$$

The stochastic interpretation of the expression in (5), for example, is that if at time t the individual subject is in S_A, the infinitestimal transition probability that, in time Δt, a change occurs in its state is:

$$\xi_{AM}\Delta t \cdot 0(\Delta t) \tag{5b}$$

From the relations given in (3) and the assumptions in (4) and (5b), it can be seen that the condition (5) implies:

$$\lim_{\Delta t \to 0} \frac{P_{AM}(t,t+\Delta t)}{\Delta t} = \xi_{AM} \tag{6}$$

$$\lim_{\Delta t \to 0} \frac{P_{AD}(t,t+\Delta t)}{\Delta t} = 0 \tag{6a}$$

Similarly, from (3) and (4), the condition in (5a) implies:

$$\lim_{\Delta t \to 0} \frac{P_{MD}(t,t+\Delta t)}{\Delta t} = \xi_{MD} \tag{7}$$

(ii) For fixed states S_A and S_M the limiting processes in (6), (6a), and (6b) are uniform with respect to S_A and S_M respectively (9).

The condition in (6a) is required for the present case since it ensures that the transition from S_A to S_D cannot occur in an infinitestimal time interval. This is consistent with our original assumption that 'alive' precedes 'moribund' and 'moribund' precedes 'dead' in the biological response and does not contradict the postulated mechanism of transition in which S_A is accessible to S_D via S_M in a finite time interval.

It may be noted that the transition intensities are not probabilities and consequently may assume any finite real values. And since the transition probabilities in (3) are assumed to be stationary, the intensities can be treated as unknown constants.

C. Transition Probability Functions

Let us now consider the application of the Chapman-Kolmogorov equations. Suppose s and t are the two specified times such that $0 \leq s < t < t + \Delta t \leq T$, where T is the total period of an experiment. Writing $\tau = t-s$, we can express the probabilities of transfers in the two intervals from s to t and from t to $(t + \Delta t)$ as follows:

$$P_{AA}(\tau + \Delta t) = P_{AA}(\tau)\, P_{AA}(\Delta t)$$

$$P_{AM}(\tau + \Delta t) = P_{AA}(\tau)\, P_{AM}(\Delta t) + P_{AM}(\tau)\, P_{MM}(\Delta t)$$

$$P_{AD}(\tau + \Delta t) = P_{AA}(\tau)\, P_{AD}(\Delta t) + P_{AM}(\tau)\, P_{MD}(\Delta t) + P_{AD}(\tau)$$

$$P_{MM}(\tau + \Delta t) = P_{MM}(\tau)\, P_{MM}(\Delta t)$$

$$P_{MD}(\tau + \Delta t) = P_{MM}(\tau)\, P_{MD}(\Delta t) + P_{MD}(\tau) \qquad (8)$$

The mathematical assumptions in the preceding section imply that the derivatives of these probabilities exist and hence the Chapman-Kolmogorov system of differential equations is as follows:

$$\frac{d\, P_{AA}(\tau)}{d\tau} = -\xi_{AM} P_{AA}(\tau)$$

$$\frac{d\, P_{AM}(\tau)}{d\tau} = \xi_{AM} P_{AA}(\tau) - \xi_{MD} P_{AM}(\tau)$$

$$\frac{d\, P_{AD}(\tau)}{d\tau} = \xi_{MD} P_{AM}(\tau)$$

$$\frac{d\, P_{MM}(\tau)}{d\tau} = -\xi_{MD} P_{MM}(\tau)$$

$$\frac{d\, P_{MD}(\tau)}{d\tau} = \xi_{MD} P_{MD}(\tau) \qquad (9)$$

As solutions of (9) we obtain the following probability functions:

$$P_{AA}(\tau) = e^{-\xi_{AM}\tau}$$

$$P_{AM}(\tau) = \frac{1}{\xi_{MD}-\xi_{AM}} \left(e^{-\xi_{AM}\tau} - e^{-\xi_{MD}\tau}\right)$$

$$P_{AD}(\tau) = \frac{1}{\xi_{MD}-\xi_{AM}} \left\{\xi_{MD}(1-e^{-\xi_{AM}\tau}) - \xi_{AM}(1-e^{-\xi_{MD}\tau})\right\}$$

$$P_{MM}(\tau) = e^{-\xi_{MD}\tau}$$

$$P_{MD}(\tau) = 1-e^{-\xi_{MD}\tau}. \tag{10}$$

It is of interest here to note the special case in which the two intensities ξ_{AM} and ξ_{MD} are equal. In this case the conditional probabilities $P_{AM}(\tau)$ and $P_{AD}(\tau)$ may be represented as follows:

$$\lim_{\xi_{MD}\to\xi_{AM}} P_{AM}(\tau) = \lim_{\xi_{MD}\to\xi_{AM}} \frac{\xi_{AM}}{\xi_{MD}-\xi_{AM}} \left(e^{-\xi_{AM}\tau} - e^{-\xi_{AM}\tau}\right) = \tau\xi_{MD}e^{-\xi_{MD}\tau}$$

$$\lim_{\xi_{MD}\to\xi_{AM}} P_{AD}(\tau) = \lim_{\xi_{MD}\to\xi_{AM}} \frac{1}{\xi_{MD}-\xi_{AM}} \left\{\xi_{MD}(1-e^{-\xi_{AM}\tau}) - \xi_{AM}(1-e^{-\xi_{MD}\tau})\right\}$$

$$= \lim_{\xi_{MD}\to\xi_{AM}} \left\{(1-e^{-\xi_{AM}\tau}) - \tau\xi_{AM}e^{-\xi_{MD}\tau}\right\}$$

$$= 1-e^{-\xi_{MD}\tau}(1+\tau\xi_{MD}) \tag{11}$$

V. ESTIMATION OF PARAMETERS

A. Probabilities of the Alternative Paths

At the beginning of the experiment all subjects to be exposed to a pre-specified dose level are in state S_A. During the course of the experiment each subject is observed at each of the pre-specified times and noted as being in one of the three states S_A, S_M, and S_D on each occasion. Accordingly each subject can be regarded as having reached its final state by traversing one of the various alternative paths exemplified for $t = 3$ in Figure 1. It is assumed that the paths are characterized by a set of stochastic or probabilistic laws which specify the probability of any particular path according to the transition intensities as previously defined.

The main purposes of this chapter are to estimate:

(a) transition intensities $\hat{\zeta}_{AM}$ and $\hat{\zeta}_{MD}$ for each dose level,

(b) ED_{50} and ET_{50} (effective dose and effective time required to produce 50 per cent affected).

Let us consider the paths through which an individual subject selected at random can reach one of the three states S_A, S_M, and S_D at t_j, $j = 1, \ldots, k$, where throughout this chapter, t_k will refer to the end of the final time interval. Denote by Q_{AA}, Q_{AMj}, Q_{ADj} and Q_{MDjh} the four mutually

exclusive and exhaustive classes defined as follows:

(a) Q_{AA}: the single path through which an individual subject reaches S_A at t_k, $(j = 1, \ldots, k)$.

(b) Q_{AMj}: the class of j paths through which an individual subject in S_A at t_{j-1} is transferred to S_M in $(t_j - t_{j-1})$ and subsequently remains in S_M until t_k, $(j = 1, \ldots, k)$.

(c) Q_{ADj}: the class of j paths through which an individual subject in S_A at t_{j-1} is transferred to S_D in $(t_j - t_{j-1})$, $(j = 1, \ldots, i)$.

(d) Q_{MDjh}: the class of $j(j-1)/2$ paths through which an individual subject in S_A at t_h is transferred to S_M in time $(t_{h+1} - t_h)$, remains in S_M up to t_{j-1} and is transferred to S_D in $(t_j - t_{j-1})$, $(h = 0, \ldots, j-2)$, $(j = 1, \ldots, k)$.

At the end of the final time interval k, the numbers of these mutually exclusive paths are therefore:

(a) 1 path in class Q_{AA}

(b) k paths in class Q_{AMj}

(c) k paths in class Q_{ADj}

(d) $k(k-1)/2$ paths in class Q_{MDjh}

giving a total of (1+k)(2+k)/2 mutually exclusive and exhaustive paths which may exist at t_k. For each dose level these paths can be exhibited as follows:

	t_0	t_1	t_2	...	t_{j-1}	t_j	...	t_{k-1}	t_k
Q_{AA}	S_A	S_A	S_A	...	S_A	S_A	...	S_A	S_A
Q_{AMj}	S_A	S_A	S_A	...	S_A	S_M	...	S_M	S_M
Q_{ADj}	S_A	S_A	S_A	...	S_A	S_D	...	S_D	S_D
	S_A	S_M	S_M	...	S_M	S_D	...	S_D	S_D
Q_{MDjh}	S_A	S_A	S_M	...	S_M	S_D	...	S_D	S_D
	.								
	.								
	.								
	S_A	S_A	S_A	...	S_M	S_D	...	S_D	S_D

The probabilities corresponding to the classes Q_{AA}, Q_{AMj}, Q_{ADj} and Q_{MDjh} respectively can then be written as:

$$\Pr\{Q_{AA}\} = \prod_{j=1}^{k} P_{AA}(t_{j-1}, t_j)$$

$$\Pr\{Q_{AMj}\} = \prod_{u=1}^{j-1} P_{AA}(t_{u-1}, t_u) P_{AM}(t_{j-1}, t_j) \prod_{u=j+1}^{k} P_{MM}(t_{u-1}, t_u)$$

$$\Pr\{Q_{ADj}\} = \prod_{u=0}^{j-1} P_{AA}(t_{u-1}, t_u) P_{AD}(t_{j-1}, t_j)$$

$$\Pr\{Q_{MDjh}\} = \prod_{u=0}^{h} P_{AA}(t_u - t_o) P_{AM}(t_h, t_{h+1}) \prod_{v=h+1}^{j-2} P_{MM}(t_v, t_{v+1}) P_{MD}(t_{j-1}, t_j)$$

(12)

Further, since the Markov process in the present study is assumed to be homogeneous in time, these probabilities can be rewritten as follows:

$$\Pr\{Q_{AA}\} = P_{AA}(t_k - t_o),$$

$$\Pr\{Q_{AMj}\} = P_{AA}(t_{j-1} - t_o) P_{AM}(t_j - t_{j-1}) P_{MM}(t_k - t_j),$$

$$\Pr\{Q_{ADj}\} = P_{AA}(t_{j-1} - t_o) P_{AD}(t_j - t_{j-1}),$$

$$\Pr\{Q_{MDjh}\} = P_{AA}(t_h - t_o) P_{AM}(t_{h+1} - t_h) P_{MM}(t_{j-1} - t_{h+1}) P_{MD}(t_j - t_{j-1})$$

(13)

Substituting the probability functions given in (10), we may now express these probabilities in terms of the transition intensities as:

$$\Pr\{Q_{AA}\} = e^{-\int_{AM}(t_k - t_o)}$$

$$\Pr\{Q_{AMj}\} = \frac{\hat{\xi}_{AM}}{\hat{\xi}_{MD}-\hat{\xi}_{AM}}\left\{e^{-\hat{\xi}_{AM}(t_j-t_{j-1})} - e^{-\hat{\xi}_{MD}(t_j-t_{j-1})}\right\} x$$

$$e^{-\hat{\xi}_{AD}(t_{j-1}-t_o) - \hat{\xi}_{MD}(t_k-t_j)} \qquad j=1, \ldots, k$$

$$\Pr\{Q_{ADj}\} = \frac{1}{\hat{\xi}_{MD}-\hat{\xi}_{AM}} e^{-\hat{\xi}_{AM}(t_{j-1}-t_o)} \left[\hat{\xi}_{MD}\left\{1-e^{-\hat{\xi}_{AM}(t_j-t_{j-1})}\right\}\right.$$

$$\left. - \hat{\xi}_{AM}\left\{1-e^{-\hat{\xi}_{MD}(t_j-t_{j-1})}\right\}\right] \qquad j=1, \ldots, k$$

$$\Pr\{Q_{MDjh}\} = \frac{\hat{\xi}_{AM}}{\hat{\xi}_{MD}-\hat{\xi}_{AM}}\left\{1-e^{-\hat{\xi}_{MD}(t_j-t_{j-1})}\right\}\left\{e^{-\hat{\xi}_{AM}(t_{h+1}-t_o)-\hat{\xi}_{MD}(t_{j-1}-t_{h+1})}\right.$$

$$\left. - e^{-\hat{\xi}_{AM}(t_h-t_o)-\hat{\xi}_{MD}(t_{j-1}-t_h)}\right\} \qquad h=0, \ldots, j-2;$$

$$j=2, \ldots, k \qquad (14)$$

B. Estimation of the Transition Intensities

Let r_{AA}, r_{AMj}, r_{ADj}, and r_{MDjh} represent the numbers of individual subjects observed in classes Q_{AA}, Q_{AMj}, Q_{ADj}, and Q_{MDjh} respectively, at the conclusion of the experiment. Then the set of random variables r_{AA}, r_{AMj}, r_{ADj}, and r_{MDjh} is multinomially distributed so that the likelihood function of the observations for a given dose level is:

$$f(r_{AA}, r_{AMj}, r_{ADj}, r_{MDjh})$$

$$= C'\left[\Pr\{Q_{AA}\}\right]^{r_{AA}} \prod_{j=1}^{k}\left[\Pr\{Q_{AMj}\}\right]^{r_{AMj}} \left[\Pr\{Q_{ADj}\}\right]^{r_{ADj}}$$

$$x \prod_{j=2}^{k}\prod_{h=0}^{j-2}\left[\Pr\{Q_{MDjh}\}\right]^{r_{MDjh}} \qquad (15)$$

where

$$C' = \frac{n_i!}{r_{AA}! \prod_{j=1}^{k} r_{AMj}! \; r_{ADj}! \prod_{j=2}^{k}\prod_{h=0}^{j-2} r_{MDjh}!}$$

The logarithm of the likelihood function is:

$$L=C+r_{AA}\log \Pr\{Q_{AA}\} + \sum_{j=1}^{k} r_{AM}\log \Pr\{Q_{AMj}\} + r_{ADj} \Pr\{Q_{ADj}\}$$

$$+ \sum_{j=2}^{k}\sum_{h=0}^{j-2} r_{MDjh} \Pr\{Q_{MDjh}\} \qquad (16)$$

in which C = log C' is a constant.

Substituting the expression for the probability functions given in (14), we may represent (16) as follows:

$$
\begin{aligned}
L = C - r_{AA}\hat{\xi}_{AM}(t_k - t_o) - \sum_{j=1}^{k} \Big[& r_{AMj}\Big\{\hat{\xi}_{AM}(t_{j-1}-t_o) + \hat{\xi}_{MD}(t_k - t_j) \\
& - \log \hat{\xi}_{AM} + \log (\hat{\xi}_{MD} - \hat{\xi}_{AM}) - \Big[\log 1 - e^{(t_j - t_{j-1})(\hat{\xi}_{AM} - \hat{\xi}_{MD})}\Big]\Big\} \\
& + r_{ADj}\Big\{\hat{\xi}_{AM}(t_{j-1}-t_o) + \log\big[(\hat{\xi}_{MD} - \hat{\xi}_{AM})\big] \\
& - \log\big[\hat{\xi}_{MD}(1 - e^{-\hat{\xi}_{AM}(t_j - t_{j-1})}) - \hat{\xi}_{AM}(1 - e^{-\hat{\xi}_{MD}(t_j - t_{j-1})})\big]\Big\}\Big] \\
& + \sum_{j=2}^{k}\sum_{h=0}^{j-2} r_{MDjh}\Big[\log\Big\{1 - e^{-\hat{\xi}_{MD}(t_j - t_{j-1})}\Big\} \\
& + \log \hat{\xi}_{AM} - \log (\hat{\xi}_{MD} - \hat{\xi}_{AM}) \\
& + \log\Big\{e^{-\hat{\xi}_{AM}(t_{h+1} - t_o) - \hat{\xi}_{MD}(t_{j-1} - t_{h+1})} \\
& - e^{-\hat{\xi}_{AM}(t_h - t_o) - \hat{\xi}_{MD}(t_{j-1} t_h)}\Big\}\Big] . \qquad (17)
\end{aligned}
$$

The estimating equations for the transition intensities ξ_{AM} and ξ_{MD} obtained by differentiations are:

$$0 = \frac{\partial L}{\partial \xi_{AMi}} = -r_{AAi}(t_k - t_o) - \sum_{j=1}^{k} \Bigg[r_{AMij} \Bigg\{ (t_{j-1} - t_o) - \frac{1}{\xi_{AMi}} - \frac{1}{\xi_{MDi} - \xi_{AMi}} + \frac{(t_j - t_{j-1})\, e^{(t_j - t_{j-1})(\xi_{AMi} - \xi_{MDi})}}{1 - e^{(t_j - t_{j-1})(\xi_{AMi} - \xi_{MDi})}} \Bigg\}$$

$$+ r_{ADij} \Bigg\{ (t_{j-1} - t_o) - \frac{1}{\xi_{MDi} - \xi_{AMi}} - \frac{(t_j - t_{j-1})\xi_{MDi}\, e^{-\xi_{AMi}(t_j - t_{j-1})} - (1 - e^{-\xi_{MDi}(t_j - t_{j-1})})}{\xi_{MDi}(1 - e^{-\xi_{MDi}(t_j - t_{j-1})}) - \xi_{AMi}(1 - e^{-\xi_{MDi}(t_j - t_{j-1})})} \Bigg\} \Bigg]$$

$$+ \sum_{j=2}^{k} \sum_{h=0}^{j-2} r_{MDjh} \Bigg\{ \frac{1}{\xi_{AMi}} + \frac{1}{\xi_{MDi} - \xi_{AMi}}$$

$$- \frac{1}{e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})} - e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)}}$$

$$x \left[(t_{h+1}-t_o) e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}t_{h+1})} \right.$$

$$\left. - (t_h-t_o) e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)} \right] \Bigg\} . \qquad (18)$$

$$0 = \frac{\partial L}{\partial \xi_{MDi}} = -\sum_{j=1}^{k} \left[r_{AMij} \left\{ (t_k-t_j) + \frac{1}{\xi_{MDi}-\xi_{AMi}} \right. \right.$$

$$\left. - \frac{(t_j-t_{j-1}) e^{(t_j-t_{j-1})(\xi_{AMi}-\xi_{MDi})}}{1-e^{(t_j-t_{j-1})(\xi_{AMi}-\xi_{MDi})}} \right\} + r_{ADij} \left\{ \frac{1}{\xi_{MDi}-\xi_{AMi}} \right.$$

$$\left. \left. - \frac{1-e^{-\xi_{AMi}(t_j-t_{j-1})} - (t_j-t_{j-1}) \xi_{AMi} e^{-\xi_{MDi}(t_j-t_{j-1})}}{\xi_{MDi}(1-e^{-\xi_{AMi}(t_j-t_{j-1})}) - \xi_{AMi}(1-e^{-\xi_{MDi}(t_j-t_{j-1})})} \right\} \right]$$

$$+ \sum_{j=2}^{k} \sum_{h=0}^{j-2} r_{MDjh} \left\{ \frac{(t_j-t_{j-1}) e^{-\xi_{MDi}(t_j-t_{j-1})}}{1-e^{-\xi_{MDi}(t_j-t_{j-1})}} - \frac{1}{\xi_{MDi}-\xi_{AMi}} \right.$$

$$- \frac{1}{e^{-\hat{\xi}_{AMi}(t_{h+1}-t_o)-\hat{\xi}_{MDi}(t_{j-1}-t_{h+1})} - e^{-\hat{\xi}_{AMi}(t_h-t_o)-\hat{\xi}_{MDi}(t_{j-1}-t_h)}}$$

$$x\Big[(t_{j-1}-t_{h+1})\, e^{-\hat{\xi}_{AMi}(t_{h+1}-t_o)-\hat{\xi}_{MDi}(t_{j-1}-t_{h+1})}$$

$$- (t_{j-1}-t_h)\, e^{-\hat{\xi}_{AMi}(t_h-t_o)-\hat{\xi}_{MDi}(t_{j-1}-t_h)}\Big]\Big\}. \qquad (19)$$

No explicit solutions for $\hat{\xi}_{AMi}$ and $\hat{\xi}_{MDi}$ can be obtained from the equations in (18) and (19) so that iterative procedures are required to obtain the estimates of the transition intensities.

The population variances of the estimates of the transition intensities can be approximated by the asymptotic variances of the estimates. Then, let

$$\hat{\xi} = \left[\hat{\xi}_{AA},\ \hat{\xi}_{AM},\ \hat{\xi}_{MM},\ \hat{\xi}_{MD}\right]$$

be a row vector whose elements are the estimated transition intensities and let

$$\xi = E\ (\hat{\xi})$$

Then the asymptotic variances and covariances of the estimated transition intensities are given by the inverse of matrix of second derivatives (cf. Cramer, 1945):

$$
-E\begin{bmatrix}
\frac{\partial^2 L}{\partial \hat{\xi}_{AA}^2} & \frac{\partial^2 L}{\partial \hat{\xi}_{AA}\,\partial \hat{\xi}_{AM}} & \frac{\partial^2 L}{\partial \hat{\xi}_{AA}\,\partial \hat{\xi}_{MM}} & \frac{\partial^2 L}{\partial \hat{\xi}_{AA}\,\partial \hat{\xi}_{MD}} \\
 & \frac{\partial^2 L}{\partial \hat{\xi}_{AM}^2} & \frac{\partial^2 L}{\partial \hat{\xi}_{AM}\,\partial \hat{\xi}_{MM}} & \frac{\partial^2 L}{\partial \hat{\xi}_{AM}\,\partial \hat{\xi}_{MD}} \\
 & & \frac{\partial^2 L}{\partial \hat{\xi}_{MM}^2} & \frac{\partial^2 L}{\partial \hat{\xi}_{MM}\,\partial \hat{\xi}_{MD}} \\
 & \text{(symmetric)} & & \frac{\partial^2 L}{\partial \hat{\xi}_{MD}^2}
\end{bmatrix} \qquad (20)
$$

The elements of the matrix are the second derivatives of L as given below.

$$
\frac{\partial^2 L}{\partial \hat{\xi}_{AMi}^2} = -\sum_{j=1}^{k}\left[r_{AMij}\left\{ \frac{1}{\hat{\xi}_{AMi}^2} - \frac{1}{(\hat{\xi}_{MDi} - \hat{\xi}_{AMi})^2} \right.\right.
$$

$$+\frac{(t_j-t_{j-1})^2\, e^{(t_j-t_{j-1})(\xi_{AMi}-\xi_{MDi})}}{1-e^{(t_j-t_{j-1})(\xi_{AMi}-\xi_{MDi})^2}}\Bigg\}+ r_{ADij}\Bigg\{-\frac{1}{(\xi_{MDi}-\xi_{AMi})^2}$$

$$+\frac{(t_j-t_{j-1})^2\,\xi_{MDi}\, e^{-\xi_{AMi}(t_j-t_{j-1})}}{\xi_{MDi}(1-e^{-\xi_{AMi}(t_j-t_{j-1})})-\xi_{AMi}(1-e^{-\xi_{MDi}(t_j-t_{j-1})})}$$

$$+\frac{\left[(t_j-t_{j-1})\xi_{MDi}e^{-\xi_{AMi}(t_j-t_{j-1})}-(1-e^{-\xi_{MDi}(t_j-t_{j-1})})\right]^2}{\left[\xi_{MDi}(1-e^{-\xi_{AMi}(t_j-t_{j-1})})-\xi_{AMi}(1-e^{-\xi_{MDi}(t_j-t_{j-1})})\right]^2}\Bigg\}\Bigg]$$

$$+\sum_{j=2}^{k}\sum_{h=0}^{j-2} r_{MDijh}\left[-\frac{1}{\xi_{AMi}^2}+\frac{1}{(\xi_{MDi}-\xi_{AMi})^2}\right.$$

$$+\frac{1}{e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})}-e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)}}$$

$$x\Big\{(t_{h+1}-t_o)^2\, e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})}$$

$$-(t_h-t_o)^2\, e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)}\Big\}$$

$$+\left\{\frac{1}{e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})}-e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)}}\right.$$

$$\times\left[(t_{h+1}-t_o)e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})}\right.$$

$$\left.\left.-(t_h-t_o)e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)}\right]\right\}^2\Bigg] \qquad (21)$$

$$\frac{\partial^2 L}{\partial \xi_{MDi}^2}=-\sum_{j=1}^{k}\left[r_{AMij}\left\{-\frac{1}{(\xi_{MDi}-\xi_{AMi})^2}\right.\right.$$

$$\left.-\frac{(t_j-t_{j-1})^2\, e^{(t_j-t_{j-1})(\xi_{AMi}-\xi_{MDi})}}{(1-e^{(t_j-t_{j-1})(\xi_{AMi}-\xi_{MDi})})^2}\right\}+r_{ADij}\left\{-\frac{1}{(\xi_{MDi}-\xi_{AMi})^2}\right.$$

$$-\frac{(t_j-t_{j-1})^2\,\xi_{AMi}\, e^{-\xi_{MDi}(t_j-t_{j-1})}}{\xi_{MDi}(1-e^{-\xi_{AMi}(t_j-t_{j-1})})-\xi_{AMi}(1-e^{-\xi_{MDi}(t_j-t_{j-1})})}$$

$$- \frac{\left[(t_j-t_{j-1})\xi_{AMi} e^{-\xi_{MDi}(t_j-t_{j-1})} - (1-e^{-\xi_{AMi}(t_j-t_{j-1})})\right]^2}{\left[\xi_{MDi}(1-e^{-\xi_{AMi}(t_j-t_{j-1})}) - \xi_{AMi}(1-e^{-\xi_{MDi}(t_j-t_{j-1})})\right]^2} \Bigg\}$$

$$+ \sum_{j=2}^{k} \sum_{h=0}^{j-2} r_{MDijh} \left[\frac{(t_j-t_{j-1})^2 e^{-\xi_{MDi}(t_j-t_{j-1})}}{(1-e^{-\xi_{MDi}(t_j-t_{j-1})})^2} + \frac{1}{(\xi_{MDi}-\xi_{AMi})^2} \right.$$

$$+ \frac{1}{e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})} - e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)}}$$

$$\times \left\{ (t_{j-1}-t_{h+1})^2 e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})} \right.$$

$$\left. - (t_{j-1}-t_h)^2 e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)} \right\}$$

$$+ \left\{ \frac{1}{e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})} - e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)}} \right.$$

$$\times \left[(t_{j-1}-t_{h+1}) e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})} \right.$$

$$- (t_{j-1}-t_h)\, e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)}\Big]\Big\}^2\Big] \qquad (22)$$

$$\frac{\partial L}{\partial \xi_{AMi}\, \partial \xi_{MDi}} = -\sum_{j=1}^{k}\Bigg[r_{AMij}\Bigg\{\frac{1}{(\xi_{MDi}-\xi_{AMi})^2}$$

$$- \frac{(t_j-t_{j-1})^2\, e^{(t_j-t_{j-1})(\xi_{AMi}-\xi_{MDi})}}{\left[1-e^{(t_j-t_{j-1})(\xi_{AMi}-\xi_{MDi})}\right]^2}\Bigg\} + r_{ADij}\Bigg\{\frac{1}{(\xi_{MDi}-\xi_{AMi})^2}$$

$$- \frac{1}{\left[\xi_{MDi}(1-e^{-\xi_{AMi}(t_j-t_{j-1})}) - \xi_{AMi}(1-e^{-\xi_{MDi}(t_j-t_{j-1})})\right]^2}$$

$$x\left[1-e^{-\xi_{AMi}(t_j-t_{j-1})} + (t_j-t_{j-1})\,\xi_{AMi}\, e^{-\xi_{AMi}(t_j-t_{j-1})}\right]$$

$$x\left[1-e^{-\xi_{MDi}(t_j-t_{j-1})}\;\; 1 - (t_j-t_{j-1})\,\xi_{MDi}\right]\Bigg\}\Bigg]$$

$$+\sum_{j=2}^{k}\sum_{h=0}^{j-2} r_{MDjh}\left[-\frac{1}{(\xi_{MDi}-\xi_{AMi})^2}\right.$$

$$+\frac{1}{e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})}-e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)}}$$

$$x\left\{(t_{h+1}-t_o)(t_{j-1}-t_{h+1})\,e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})}\right.$$

$$\left.-(t_h-t_o)(t_{j-1}-t_h)\,e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)}\right\}$$

$$-\frac{1}{\left\{e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})}-e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)}\right\}^2}$$

$$x\left\{(t_{h+1}-t_o)\,e^{-\xi_{AMi}(t_{h+1}-t_o)-\xi_{MDi}(t_{j-1}-t_{h+1})}\right.$$

$$\left.-(t_h-t_o)\,e^{-\xi_{AMi}(t_h-t_o)-\xi_{MDi}(t_{j-1}-t_h)}\right\}$$

$$x \left\{ (t_{j-1}-t_h)\, e^{-\hat{\xi}_{AMi}(t_h-t_o)-\hat{\xi}_{MDi}(t_{j-1}-t_h)} \right.$$

$$\left. - (t_{j-1}-t_{h+1})\, e^{-\hat{\xi}_{AMi}(t_{h+1}-t_o)-\hat{\xi}_{MDi}(t_{j-1}-t_{h+1})} \right\} \Bigg]. \qquad (23)$$

Since the intensities $\hat{\xi}_{AMi}$ and $\hat{\xi}_{MDi}$ are themselves unknown constants, numerical values for the approximate asymptotic variances and covariances are obtained by substituting in (21), (22) and (23) the estimates of $\hat{\xi}_{AMi}$ and $\hat{\xi}_{MDi}$ from (18) and (19). Numerical example illustrating the basic procedure is given in chapter 6.

C. Estimation of ED_{50} and ET_{50}

In the foregoing sections the experimental situation has been specified as a homogeneous Markov process and methods for estimating the corresponding transition intensities have been obtained. For application in biological assay it is now necessary to obtain a relevant model which specifies the usual bioassay concepts of ED_{50} and ET_{50} in terms of these transition intensities. Additional considerations to this end will now be discussed.

1. Assumptions

In many cases of the quantal response bioassay analysis, it has been demonstrated that the dosage-response relationship can be very conveniently represented by the logistic function (19), (30). It has been pointed out previously that we have assumed that the present Markov process is time homogeneous. Consistently with this it will now be assumed that the probability that a subject observed to be in one state at any time s will be observed in another state at a later time t can be expressed in terms of the logistic function.

2. Relation between the two models

On the basis of the preceding assumptions, the inter-relations between the stochastic process and the logistic function models are now obtained.

Because the present Markov model is assumed to be homogeneous, we may, without loss of generality, take the time interval (t-s) to be unity. Applying the logistic function to express the probability of the change of the state from 'alive' to 'affected' ('moribund or dead'), together with the probability functions in (10), we have

$$P_{AM}(1) + P_{AD}(1) = \left\{1 + e^{-(\alpha + \beta x)}\right\}^{-1} \qquad (24)$$

in which α and β are unknown constants and x is a suitable dose metameter such as the log dose. Further, since,

$$P_{AM}(1) + P_{AD}(1) = 1 - P_{AA}(1),$$

and

$$P_{AA}(1) = e^{-\xi_{AM}},$$

(24) can be rewritten as follows:

$$e^{-\xi_{AM}} = (1 + e^{\alpha + \beta x})^{-1} \tag{25}$$

Similarly for the change of state from S_M to S_D, we have,

$$P_{MD}(1) = \left\{1 + e^{-(\gamma + \delta x)}\right\}^{-1}, \tag{26}$$

in which γ and δ are unknown parameters.

And again, since

$$P_{MD}(1) = 1 - P_{MM}(1),$$

and

$$P_{MM}(1) = e^{-\xi_{MD}},$$

(26) can be rewritten as follows:

$$e^{-\xi_{MD}} = (1 + e^{\gamma + \delta x})^{-1} \tag{27}$$

From the relations in (25) and (27) it can be seen that the transition intensities have now been expressed as functions of dosage x only, a fact which is consistent and reasonable in the present bioassay context.

3. Estimates of ED_{50}

Let $ED_{50}(D)$ be the dose which produces 50 per cent 'dead' and let $ED_{50}(D+M)$ be the dose for which 50 per cent 'dead or moribund' is produced. Then, from (14), $ED_{50}(D)$ is the dosage x for which:

$$\Pr\{Q_{AA}\} + \sum_{j=1}^{k} \Pr\{Q_{AMj}\} = \tfrac{1}{2},$$

where, from (14), (25), and (27),

$$\Pr\{Q_{AA}\} = (1 + e^{\alpha + \beta x})^{-T},$$

and

$$\Pr\{Q_{AMj}\} = \frac{\log(1+e^{\alpha+\beta x})}{\log(1+e^{\gamma+\delta x}) - \log(1+e^{\alpha+\beta x})}$$

$$x \left\{ (1+e^{\alpha+\beta x})^{-(t_j-t_o)} (1+e^{\gamma+\delta x})^{-(t_k-t_j)} \right.$$

$$- (1+e^{\alpha+\beta x_j})^{-(t_{j-1}-t_o)} (1+e^{\gamma+\delta x_j})^{-(t_k-t_{j-1})} \Big\} . \qquad (28)$$

Similarly the $ED_{50}(D+M)$ is that dosage x which satisfies the relation:

$$Pr\{Q_{AA}\} = \tfrac{1}{2},$$

where, from (14) and (25),

$$Pr\{Q_{AA}\} = (1 + e^{\alpha+\beta x})^{-T} \qquad (29)$$

In this case the two previous equations can be combined to show that we can estimate $ED_{50}(D+M)$ as:

$$\hat{x}_{50}\ (D+M) = \frac{\log_e (2^{\frac{1}{T}} - 1) - \hat{\alpha}}{\hat{\beta}} \qquad (30)$$

where $\hat{\alpha}$ and $\hat{\beta}$ are the estimates of α and β respectively.

The estimation of the $ED_{50}(D)$ in (28) requires:

(a) substitution of the estimates $\hat{\alpha}$, $\hat{\beta}$, $\hat{\gamma}$, and $\hat{\delta}$, and

(b) iterations since the solution for x is not explicit.

Detailed procedures for estimating the parameters α, β, γ, and δ will be presented in subsection (5) in this chapter.

4. Estimates of ET_{50}

Whilst the preceding procedures can be used to obtain the usual bioassay quantity, ED_{50}, the present development is, however, of further value because it can be used to obtain alternative information of use in the bioassay context. Thus, for a given dose level, it is now possible to estimate the time period required to produce 50 per cent affected. For this it should be noted that the assumption that the transition probabilities are logistic is not now required because ET_{50} can be estimated for any individual fixed dose level.

Let $ET_{50}(D)$ be the time period required to produce 50 per cent 'dead' and let $ET_{50}(D+M)$ be the time period in which 50 per cent 'dead or moribund' is produced under a given dosage x. Then $ET_{50}(D)$ is the time period $(t_u - t_o)$ for which:

$$\Pr\{Q_{AA}\} + \sum_{j=1}^{u} \Pr\{Q_{AMj}\} = \tfrac{1}{2},$$

where from (14),

$$\Pr\{Q_{AA}\} = e^{-\hat{\xi}_{AM}(t_u - t_o)}$$

and

$$\Pr\{Q_{AMj}\} = \frac{\hat{\xi}_{AM}}{\hat{\xi}_{MD} - \hat{\xi}_{AM}} e^{-\hat{\xi}_{AM}(t_{j-1} - t_o) - \hat{\xi}_{MD}(t_u - t_j)}$$

$$x \left\{ e^{-\hat{\xi}_{AM}(t_j - t_{j-1})} - e^{-\hat{\xi}_{MD}(t_j - t_{j-1})} \right\}. \qquad (31)$$

Again an iterative procedure is required for the solution. Similarly the $ET_{50}(D+M)$ is the time period for which:

$$Pr\{Q_{AA}\} = \tfrac{1}{2},$$

where

$$Pr\{Q_{AA}\} = e^{-\hat{\xi}_{AM}(t_u - t_o)} \qquad (32)$$

From the last two equations combined, the $ET_{50}(D+M)$ can then be estimated explicitly from the relationship

$$\hat{t}_{50}(D+M) = \frac{\log e^2}{\hat{\xi}_{AM}},$$

where $\hat{\xi}_{AM}$ is the maximum likelihood estimate of ξ_{AM} obtained from Equations (18) and (19).

5. Maximum likelihood estimates of the parameters

Substituting the expressions in (25) and (27) for the transition intensities in (17) and summing over all dose levels, we obtain the likelihood function for the parameters as follows:

$$L = C + \sum_{i=1}^{m} \Bigg[- r_{AAi}(t_k - t_o) \log (1+e^{\alpha+\beta x_i})$$

$$- \sum_{j=1}^{k} r_{AMij} \Big\{ (t_{j-1} - t_o) \log (1+e^{\alpha+\beta x_i}) + (t_k - t_j) \log (1+e^{\gamma+\delta x_i})$$

$$- \log \log (1+e^{\alpha+\beta x_i}) + \log \log \frac{1+e^{\gamma+\delta x_i}}{1+e^{\alpha+\beta x_i}}$$

$$- \log \Big[1 - (1+e^{\alpha+\beta x_i})^{t_j - t_{j-1}} (1+e^{\gamma+\delta x_i})^{-(t_j - t_{j-1})} \Big] \Big\}$$

$$- \sum_{j=1}^{k} r_{ADij} \Big\{ (t_{j-1} - t_o) \log (1+e^{\alpha+\beta x_i}) + \log \log \frac{1+e^{\gamma+\delta x_i}}{1+e^{\alpha+\beta x_i}}$$

$$- \log \Big[\log (1+e^{\gamma+\delta x_i}) \Big(1 - (1+e^{\alpha+\beta x_i})^{-(t_j - t_{j-1})} \Big)$$

$$- \log (1+e^{\alpha+\beta x_i}) \Big(1 - (1+e^{\gamma+\delta x_i})^{-t_j - t_{j-1}} \Big) \Big] \Big\}$$

$$+ \sum_{j=2}^{k} \sum_{h=0}^{j-2} r_{MDijh} \Big\{ \log \Big[1 - (1+e^{\gamma+\delta x_i})^{-(t_j - t_{j-1})} \Big]$$

$$+ \log \log (1+e^{\alpha+\beta x_i}) - \log \log \frac{1+e^{\gamma+\delta x_i}}{1+e^{\alpha+\beta x_i}}$$

$$+ \log \Big[(1+e^{\alpha+\beta x_i})^{-(t_{h+1}-t_o)} (1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_{h+1})}$$

$$- (1+e^{\alpha+\beta x_i})^{-(t_h-t_o)} (1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_h)} \Big] \Big\} \Big] . \qquad (33)$$

From this, the estimating equations for the parameters α and γ can be obtained, as usual, by differentiation to give:

$$0 = \frac{\partial L}{\partial \alpha} = \sum_{i=1}^{m} e^{\alpha+\beta x_i} \Bigg[- \frac{r_{AAi}(t_k-t_o)}{1+e^{\alpha+\beta x_i}} - \sum_{j=1}^{k} r_{AMij} \Bigg\{ \frac{t_{j-1}-t_o}{1+e^{\alpha+\beta x_i}}$$

$$- \frac{1}{\log(1+e^{\alpha+\beta x_i})(1+e^{\alpha+\beta x_i})}$$

$$- \frac{1}{\Big[\log(1+e^{\gamma+\delta x_i}) - \log(1+e^{\alpha+\beta x_i})\Big](1+e^{\alpha+\beta x_i})}$$

$$+ \frac{(t_j-t_{j-1})(1+e^{\alpha+\beta x_i})^{t_j-t_{j-1}-1}(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}}{1-(1+e^{\alpha+\beta x_i})^{t_j-t_{j-1}}(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}} \Bigg\}$$

$$-\sum_{j=1}^{k} r_{ADij}\left\{\frac{t_{j-1}-t_o}{1+e^{\alpha+\beta x_i}}\right.$$

$$-\frac{1}{\left[\log\,(1+e^{\gamma+\delta x_i}) - \log\,(1+e^{\alpha+\beta x_i})\right](1+e^{\alpha+\beta x_i})}$$

$$-\frac{1}{\left(\begin{array}{l}\log\,(1+e^{\gamma+\delta x_i})\left[1-(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})}\right] \\ \\ -\log\,(1+e^{\alpha+\beta x_i})\left[1-(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}\right]\end{array}\right)}$$

$$x\left[\log\,(1+e^{\gamma+\delta x_i})(t_j-t_{j-1})(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})-1}\right.$$

$$\left.-\left(1-(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}\right)(1+e^{\alpha+\beta x_i})^{-1}\right]\Bigg\}$$

$$+\sum_{j=2}^{k}\sum_{h=0}^{j-2} r_{MDijh}\left\{\frac{1}{\log\,(1+e^{\alpha+\beta x_i})(1+e^{\alpha+\beta x_i})}\right.$$

$$+\frac{1}{\left[\log\,(1+e^{\gamma+\delta x_i}) - \log\,(1+e^{\alpha+\beta x_i})\right](1+e^{\alpha+\beta x_i})}$$

$$- \frac{1}{\left((1+e^{\alpha+\beta x_i})^{-(t_{h+1}-t_o)} (1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_{h+1})} \right)}$$

$$\left(- (1+e^{\alpha+\beta x_i})^{-(t_h-t_o)} (1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_h)} \right)$$

$$x \left[(t_{h+1}-t_o)(1+e^{\alpha+\beta x_i})^{-(t_{h+1}-t_o)-1} (1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_{h+1})} \right.$$

$$\left. - (t_h-t_o)(1+e^{\alpha+\beta x_i})^{-(t_h-t_o)-1} (1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_h)} \right] \Bigg\}. \qquad (34)$$

$$0 = \frac{\partial L}{\partial \gamma} = \sum_{i=1}^{m} e^{\gamma+\delta x_i} \left[- \sum_{j=1}^{k} r_{AMij} \left\{ \frac{t_k-t_o}{1+e^{\gamma+\delta x_i}} \right.\right.$$

$$+ \frac{1}{\left[\log (1+e^{\gamma+\delta x_i}) - \log (1+e^{\alpha+\beta x_i}) \right] (1+e^{\gamma+\delta x_i})}$$

$$\left.\left. - \frac{(t_j-t_{j-1})(1+e^{\alpha+\beta x_i})^{t_j-t_{j-1}} (1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})-1}}{1 - (1+e^{\alpha+\beta x_i})^{t_j-t_{j-1}} (1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}} \right\} \right.$$

$$\left. - \sum_{j=1}^{k} r_{ADij} \left\{ \frac{1}{\left[\log (1+e^{\gamma+\delta x_i}) - \log (1+e^{\alpha+\beta x_i}) \right] (1+e^{\gamma+\delta x_i})} \right. \right.$$

$$- \frac{1}{\begin{pmatrix} \log\,(1+e^{\gamma+\delta x_i})\left[1-(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})}\right] \\ -\log\,(1+e^{\alpha+\beta x_i})\left[1-(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}\right] \end{pmatrix}}$$

$$\times\left[(1+e^{\gamma+\delta x_i})^{-1}\left(1-(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})}\right)\right.$$

$$\left.-\log\,(1+e^{\alpha+\beta x_i})(t_j-t_{j-1})(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})-1}\right]\Bigg\}$$

$$+\sum_{j=2}^{k}\sum_{h=0}^{j-2} r_{MDijh}\left\{\frac{(t_j-t_{j-1})(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})-1}}{1-(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}}\right.$$

$$- \frac{1}{\left[\log\,(1+e^{\gamma+\delta x_i})-\log\,(1+e^{\alpha+\beta x_i})\right](1+e^{\gamma+\delta x_i})}$$

$$- \frac{1}{\begin{pmatrix} (1+e^{\alpha+\beta x_i})^{-(t_{h+1}-t_o)}(1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_{h+1})} \\ -(1+e^{\alpha+\beta x_i})^{-(t_h-t_o)}(1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_h)} \end{pmatrix}}$$

$$x\left[(1+e^{\alpha+\beta x_i})^{-(t_{h+1}-t_o)}(t_{j-1}-t_{h+1})(1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_{h+1})-1}\right.$$

$$\left.-\ (1+e^{\alpha+\beta x_i})^{-(t_h-t_o)}(t_{j-1}-t_h)(1+e^{\gamma+\delta x_i})^{-(t_{j-1}t_h)-1}\right]\Bigg\} \qquad (35)$$

Let L_i denote the likelihood function for dose i which can be obtained by substituting the expressions in (25) and (27) for the transition intensities in (17). Then the estimating equations for the parameters β and δ can be expressed as follows:

$$0 = \frac{\partial L}{\partial \beta} = \sum_{i=1}^{m}\left(\frac{\partial L_i}{\partial \alpha}\right) x_i$$

$$0 = \frac{\partial L}{\partial \delta} = \sum_{i=1}^{m}\left(\frac{\partial L_i}{\partial \gamma}\right) x_i \qquad (36)$$

Since explicit solutions for the parameters α, β, γ, and δ cannot be obtained from the estimating equations above, iterative procedures are required to determine their estimates.

To obtain the asymptotic variances and covariances

$$\hat{\alpha},\ \hat{\beta},\ \hat{\gamma},\ \text{and}\ \hat{\delta}$$

the second derivatives given below are first required.

$$\frac{\partial^2 L}{\partial \alpha^2} = \sum_{i=1}^{m} e^{2(\alpha+\beta x_i)} \left[\frac{r_{AAi}\,(t_k - t_o)}{(1+e^{\alpha+\beta x_i})^2} - \sum_{j=1}^{k} r_{AMij} \left\{ \frac{-(t_{j-1}-t_o)}{(1+e^{\alpha+\beta x_i})^2} \right.\right.$$

$$+ \frac{\log\,(1+e^{\alpha+\beta x_i}) + 1}{\left[\log\,(1+e^{\alpha+\beta x_i})\,(1+e^{\alpha+\beta x_i})\right]^2}$$

$$+ \frac{\log\,(1+e^{\gamma+\delta x_i}) - \log\,(1+e^{\alpha+\beta x_i}) - 1}{\left[\log\,(1+e^{\gamma+\delta x_i}) - \log\,(1+e^{\alpha+\beta x_i})\right]^2 (1+e^{\alpha+\beta x_i})^2}$$

$$+ \frac{1}{1 - (1+e^{\alpha+\beta x_i})^{t_j - t_{j-1}} (1+e^{\gamma+\delta x_i})^{-(t_j - t_{j-1})}}$$

$$x \left[(t_j - t_{j-1})(t_j - t_{j-1} - 1) \right]$$

$$x \left[(1+e^{\alpha+\beta x_i})^{t_j - t_{j-1} - 2} (1+e^{\gamma+\delta x_i})^{-(t_j - t_{j-1})} \right]$$

$$\left. \left. + \frac{(t_j - t_{j-1})^2 (1+e^{\alpha+\beta x_i})^{2(t_j - t_{j-1} - 1)} (1+e^{\gamma+\delta x_i})^{-2(t_j - t_{j-1})}}{\left[1 - (1+e^{\alpha+\beta x_i})^{t_j - t_{j-1}} (1+e^{\gamma+\delta x_i})^{-(t_j - t_{j-1})}\right]^2} \right\} \right]$$

$$-\sum_{j=1}^{k} r_{ADij}\left\{ -\frac{t_{j-1}-t_o}{(1+e^{\alpha+\beta x_i})^2}\right.$$

$$+\frac{\log\,(1+e^{\gamma+\delta x_i}) - \log\,(1+e^{\alpha+\beta x_i}) - 1}{\left[\log\,(1+e^{\gamma+\delta x_i}) - \log\,(1+e^{\alpha+\beta x_i})\right]^2\,(1+e^{\alpha+\beta x_i})^2}$$

$$+\frac{1}{\begin{pmatrix}\log\,(1+e^{\gamma+\delta x_i})\left[1-(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})}\right] \\ -\log\,(1+e^{\alpha+\beta x_i})\left[1-(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}\right]\end{pmatrix}}$$

$$x\left\{(t_j-t_{j-1})(t_j-t_{j-1}+1)(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})-2}\log\,(1+e^{\gamma+\delta x_i})\right.$$

$$\left.-\left(1-(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}\right)(1+e^{\alpha+\beta x_i})^{-2}\right]$$

$$+\frac{1}{\begin{pmatrix}\left[\log\,(1+e^{\gamma+\delta x_i})\left(1-(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})}\right)\right. \\ \left.-\log\,(1+e^{\alpha+\beta x_i})\left(1-(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}\right)\right]^2\end{pmatrix}}$$

$$x \left[\log (1+e^{\gamma+\delta x_i})(t_j-t_{j-1})(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})-1} \right.$$

$$\left. - \left(1 - (1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}\right)(1+e^{\alpha+\beta x_i})^{-1} \right]$$

$$x \left[(t_j-t_{j-1})(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})-1} \log (1+e^{\gamma+\delta x_i}) \right.$$

$$\left. - (1+e^{\alpha+\beta x_i})^{-1}\left(1 - (1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}\right)\right] \Bigg\}$$

$$+ \sum_{j=2}^{k} \sum_{h=0}^{j-2} r_{MDijh} \left\{ \frac{- \log (1+e^{\alpha+\beta x_i}) - 1}{\left[\log (1+e^{\alpha+\beta x_i})(1+e^{\alpha+\beta x_i})\right]^2} \right.$$

$$\left. - \frac{\log (1+e^{\gamma+\delta x_i}) - \log (1+e^{\alpha+\beta x_i}) - 1}{\left[\log (1+e^{\gamma+\delta x_i}) - \log (1+e^{\alpha+\beta x_i})\right]^2 (1+e^{\alpha+\beta x_i})^2} \right.$$

$$\left. - \frac{1}{\left(\begin{array}{l} (1+e^{\alpha+\beta x_i})^{-(t_{k+1}-t_o)} (1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_{h+1})} \\ \\ - (1+e^{\alpha+\beta x_i})^{-(t_h-t_o)} (1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_h)} \end{array} \right)} \right.$$

$$x \left[(t_{h+1}-t_o)(t_{h+1}-t_o+1) \right.$$

$$x \quad (1+e^{\alpha+\beta x_i)-(t_{h+1}-t_o)-2} (1+e^{\gamma+\delta x_i)-(t_{j-1}-t_{h+1})}$$

$$\left. - \quad (t_h-t_o)(t_h-t_o+1)(1+e^{\alpha+\beta x_i)-(t_h-t_o)-2} (1+e^{\gamma+\delta x_i)-(t_{j-1}-t_h)} \right]$$

$$x \left[-(t_{h+1}-t_o)(1+e^{\alpha+\beta x_i)-(t_{h+1}-t_o)-1} (1+e^{\gamma+\delta x_i)-(t_{j-1}-t_{h+1})} \right.$$

$$\left. +(t_h-t_o)(1+e^{\alpha+\beta x_i)-(t_h-t_o)-1} (1+e^{\gamma+\delta x_i)-(t_{j-1}-t_h)} \right]$$

$$+ \frac{1}{\left(\left[(1+e^{\alpha+\beta x_i)-(t_{h+1}-t_o)} (1+e^{\gamma+\delta x_i)-(t_{j-1}-t_{h+1})} \right. \right.$$

$$\left. \left. - (1+e^{\alpha+\beta x_i)-(t_h-t_o)} (1+e^{\gamma+\delta x_i)-(t_{j-1}-t_h)} \right]^2 \right)}$$

$$x \left[(t_{h+1}-t_o)(1+e^{\alpha+\beta x_i)-(t_{h+1}-t_o)-1} (1+e^{\gamma+\delta x_i)-(t_{j-1}-t_{h+1})} \right.$$

$$\left. \left. \left. - \quad (t_h-t_o)(1+e^{\alpha+\beta x_i)-(t_h-t_o)-1} (1+e^{\gamma+\delta x_i)-(t_{j-1}-t_h)} \right] \right\} \right] \qquad (37)$$

$$\frac{\partial^2 L}{\partial \gamma^2} = \sum_{i=1}^{m} e^{2(\gamma+\delta x_i)} \left[-\sum_{j=1}^{k} r_{AMij} \left\{ \frac{-(t_k - t_o)}{(1+e^{\gamma+\delta x_i})^2} \right.\right.$$

$$- \frac{\log (1+e^{\gamma+\delta x_i}) - \log (1+e^{\alpha+\beta x_i}) + 1}{\left[\log (1+e^{\gamma+\delta x_i}) - \log (1+e^{\alpha+\beta x_i})\right]^2 (1+e^{\gamma+\delta x_i})^2}$$

$$+ \frac{(t_j - t_{j-1})^2 (1+e^{\alpha+\beta x_i})^{t_j - t_{j-1}} (1+e^{\gamma+\delta x_i})^{-(t_j - t_{j-1})-2}}{1 - (1+e^{\alpha+\beta x_i})^{t_j - t_{j-1}} (1+e^{\gamma+\delta x_i})^{-(t_j - t_{j-1})}}$$

$$\left. + \frac{(t_j - t_{j-1})^2 (1+e^{\alpha+\beta x_i})^{2(t_j - t_{j-1})} (1+e^{\gamma+\delta x_i})^{-2(t_j - t_{j-1}+1)}}{\left[1 - (1+e^{\alpha+\beta x_i})^{t_j - t_{j-1}} (1+e^{\gamma+\delta x_i})^{-(t_j - t_{j-1})}\right]^2} \right\}$$

$$- \sum_{j=1}^{k} r_{ADij} \left\{ - \frac{\log (1+e^{\gamma+\delta x_i}) - \log (1+e^{\alpha+\beta x_i}) + 1}{\left[\log (1+e^{\gamma+\delta x_i}) - \log (1+e^{\alpha+\beta x_i})\right]^2 (1+e^{\gamma+\delta x_i})^2} \right.$$

$$+ \frac{1}{\left(\begin{array}{l} \log (1+e^{\gamma+\delta x_i}) \left[1 - (1+e^{\alpha+\beta x_i})^{-(t_j - t_{j-1})}\right] \\ \\ - \log (1+e^{\alpha+\beta x_i}) \left[1 - (1+e^{\gamma+\delta x_i})^{-(t_j - t_{j-1})}\right] \end{array} \right)}$$

$$x \left[(1+e^{\gamma+\delta x_i})^{-2} \left(1 - (1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})} \right) \right.$$

$$\left. - (t_j-t_{j-1})(t_j-t_{j-1}+1) \log (1+e^{\alpha+\beta x_i})(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})-2} \right]$$

$$+ \frac{1}{\left[\log (1+e^{\gamma+\delta x_i}) \left(1 - (1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})} \right) \right.}$$

$$\left. - \log (1+e^{\alpha+\beta x_i}) \left(1 - (1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})} \right) \right]^2$$

$$x \left[(1+e^{\gamma+\delta x_i})^{-1} \; (1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})} \right.$$

$$\left. - \log (1+e^{\alpha+\beta x_i})^{(t_j-t_{j-1})} (1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})-1} \right]$$

$$x \left[(1+e^{\gamma+\delta x_i}) \left(1 - (1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})} \right) \right.$$

$$\left. + (t_j-t_{j-1}) \log (1+e^{\alpha+\beta x_i})(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})-1} \right] \Bigg\}$$

$$+ \sum_{j=2}^{k} \sum_{h=0}^{j-2} r_{MDijh} \left\{ \frac{-(t_j-t_{j-1})(t_j-t_{j-1}+1)(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})-2}}{1 - (1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}} \right.$$

$$- \frac{(t_j - t_{j-1})^2 (1+e^{\gamma+\delta x_i})^{-2(t_j - t_{j-1}+1)}}{\left[1 - (1+e^{\gamma+\delta x_i})^{-(t_j - t_{j-1})}\right]^2}$$

$$+ \frac{\log (1+e^{\gamma+\delta x_i}) - \log (1+e^{\alpha+\beta x_i}) + 1}{\left[\log (1+e^{\gamma+\delta x_i}) - \log (1+e^{\alpha+\beta x_i})\right]^2 (1+e^{\gamma+\delta x_i})^2}$$

$$- \frac{1}{\left((1+e^{\alpha+\beta x_i})^{-(t_{h+1} - t_o)} (1+e^{\gamma+\delta x_i})^{-(t_{j-1} - t_{h+1})} \right.$$

$$\left. - (1+e^{\alpha+\beta x_i})^{-(t_h - t_o)} (1+e^{\gamma+\delta x_i})^{-(t_{j-1} - t_h)} \right)}$$

$$x \left[(t_{j-1} - t_h)(1+e^{\gamma+\delta x_i})^{-(t_{j-1} - t_h)-2} \right.$$

$$x \quad (t_{j-1} - t_{h+1})(1+e^{\alpha+\beta x_i})^{-(t_h - t_o)}$$

$$- \quad (t_{j-1} - t_{h+1})(t_{j-1} - t_{h+1}+1)(1+e^{\alpha+\beta x_i})^{-(t_{h+1} - t_o)}$$

$$\left. x \quad (1+e^{\gamma+\delta x_i})^{-(t_{j-1} - t_{h+1})-2} \right]$$

$$+\frac{1}{\left(\left[(1+e^{\alpha+\beta x_i})^{-(t_{h+1}-t_o)}(1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_{h+1})}\right.\right.}$$

$$\left.\left.-(1+e^{\alpha+\beta x_i})^{-(t_h-t_o)}(1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_h)}\right]^2\right)$$

$$x\left[(1+e^{\alpha+\beta x_i})^{-(t_{h+1}-t_o)}(t_{j-1}-t_{h+1})(1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_{h+1})-1}\right.$$

$$\left.-(1+e^{\alpha+\beta x_i})^{-(t_h-t_o)}(t_{j-1}-t_h)(1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_h)-1}\right]$$

$$x\left[(t_{j-1}-t_h)(1+e^{\alpha+\beta x_i})^{-(t_h-t_o)}(1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_h)-1}\right.$$

$$\left.\left.-(t_{j-1}-t_{h+1})(1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_{h+1})-1}(1+e^{\alpha+\beta x_i})^{-(t_{h+1}-t_o)}\right]\right\}\Bigg] \tag{38}$$

$$\frac{\partial^2 L}{\partial\alpha\partial\gamma} = \sum_{i=1}^{m} e^{\gamma+\delta x_i}\left[\sum_{j=1}^{k} r_{AMij}\left\{\frac{-1}{\left(\left[\log(1+e^{\gamma+\delta x_i})-\log(1+e^{\alpha+\beta x_i})\right]^2\right.}\right.\right.$$

$$\left.\left.\left.x\,(1+e^{\alpha+\beta x_i})(1+e^{\gamma+\delta x_i})\right)\right.\right.$$

$$+\frac{(t_j-t_{j-1})^2(1+e^{\alpha+\beta x_i})^{t_j-t_{j-1}-1}(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})-1}}{\left[1-(1+e^{\alpha+\beta x_i})^{t_j-t_{j-1}}(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})-1}\right]^2}$$

$$+\sum_{j=1}^{k} r_{ADij}\left\{-\frac{1}{\left[\log(1+e^{\gamma+\delta x_i})-\log(1+e^{\alpha+\beta x_i})\right]^2(1+e^{\alpha+\beta x_i})(1+e^{\gamma+\delta x_i})}\right.$$

$$+\frac{1}{\begin{pmatrix}\log(1+e^{\gamma+\delta x_i})\left[1-(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})}\right]\\ -\log(1+e^{\alpha+\beta x_i})\left[1-(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}\right]\end{pmatrix}}$$

$$x\left[(t_j-t_{j-1})(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})-1}(1+e^{\gamma+\delta x_i})^{-1}\right.$$

$$\left.-(t_j-t_{j-1})(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})-1}(1+e^{\alpha+\beta x_i})^{-1}\right]$$

$$-\frac{1}{\begin{pmatrix}\log(1+e^{\gamma+\delta x_i})\left[1-(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})}\right]\\ -\log(1+e^{\alpha+\beta x_i})\left[1-(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}\right]^2\end{pmatrix}}$$

$$x \left[\log (1+e^{\gamma+\delta x_i})(t_j-t_{j-1})(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})-1} \right.$$

$$\left. - \left(1 - (1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})}\right)(1+e^{\alpha+\beta x_i})^{-1} \right]$$

$$x \left[-(1+e^{\gamma+\delta x_i})^{-1}(t_j-t_{j-1})(1+e^{\alpha+\beta x_i})^{-(t_j-t_{j-1})} \right.$$

$$\left. - \log (1+e^{\alpha+\beta x_i})(t_j-t_{j-1})(1+e^{\gamma+\delta x_i})^{-(t_j-t_{j-1})-1} \right] \Bigg\}$$

$$+ \sum_{j=2}^{k} \sum_{h=0}^{j-2} r_{MDijh} \left\{ - \frac{1}{\left(\left[\log (1+e^{\gamma+\delta x_i}) - \log (1+e^{\alpha+\beta x_i}) \right]^2 \right.} \right.$$

$$\left. \left. x \, (1+e^{\alpha+\beta x_i})(1+e^{\gamma+\delta x_i}) \right) \right.$$

$$+ \frac{1}{\left((1+e^{\alpha+\beta x_i})^{-(t_{h+1}-t_o)} (1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_{h+1})} \right.}$$

$$\left. \left. - (1+e^{\alpha+\beta x_i})^{-(t_h-t_o)} (1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_h)} \right) \right.$$

$$x \left[(t_{h+1}-t_o)(t_{j-1}-t_{h+1})(1+e^{\alpha+\beta x_i})^{-(t_{h+1}-t_o)-1} \right.$$

$$\left. x \, (1+e^{\gamma+\delta x_i})^{-(t_{j-1}-t_{h+1})-1} \right.$$

$$+ (t_h-t_o)(t_{j-1}-t_h)(1+e^{\alpha+\beta x_i)-(t_h-t_o)-1}{}_{(1+e}{}^{\gamma+\delta x_i)-(t_{j-1}-t_h)}\Big]$$

$$+ \frac{1}{\left(\Big[(1+e^{\alpha+\beta x_i)-(t_{h+1}-t_o)}{}_{(1+e}{}^{\gamma+\delta x_i)-(t_{j-1}-t_{h+1})} - (1+e^{\alpha+\beta x_i)-(t_h-t_o)}{}_{(1+e}{}^{\gamma+\delta x_i)-(t_{j-1}-t_h)}\Big]^2\right)}$$

$$x\Big[(t_{h+1}-t_o)(1+e^{\alpha+\beta x_i)-(t_{h+1}-t_o)-1}{}_{(1+e}{}^{\gamma+\delta x_i)-(t_{j-1}-t_{h+1})}$$

$$- (t_h-t_o)(1+e^{\alpha+\beta x_i)-(t_h-t_o)-1}{}_{(1+e}{}^{\gamma+\delta x_i)-(t_{j-1}-t_h)}\Big]$$

$$x\Big[(t_{j-1}-t_{h+1})(1+e^{\alpha+\beta x_i)-(t_{h+1}-t_o)}{}_{(1+e}{}^{\gamma+\delta x_i)-(t_{j-1}-t_{h+1})-1}$$

$$+ (t_{j-1}-t_h)(1+e^{\alpha+\beta x_i)-(t_h-t_o)}{}_{(1+e}{}^{\gamma+\delta x_i)-(t_{j-1}-t_h)-1}\Big]\Big\}\Big] \quad (39)$$

$$\frac{\partial^2 L}{\partial \beta^2} = \sum_{i=1}^{m}\left(\frac{\partial^2 L_i}{\partial \alpha^2}\right) x_i^2$$

$$\frac{\partial^2 L}{\partial \delta^2} = \sum_{i=1}^{m}\left(\frac{\partial^2 L_i}{\partial \gamma^2}\right) x_i^2$$

$$\frac{\partial^2 L}{\partial \alpha \partial \beta} = \sum_{i=1}^{m} \left(\frac{\partial^2 L_i}{\partial \alpha^2} \right) x_i$$

$$\frac{\partial^2 L}{\partial \gamma \partial \delta} = \sum_{i=1}^{m} \left(\frac{\partial^2 L_i}{\partial \gamma^2} \right) x_i$$

$$\frac{\partial^2 L}{\partial \alpha \partial \delta} = \frac{\partial^2 L}{\partial \beta \partial \gamma} = \sum_{i=1}^{m} \left(\frac{\partial^2 L_i}{\partial \alpha \partial \gamma} \right) x_i \qquad (40)$$

The estimated asymptotic variances and covariances can then be obtained by replacing the parameters in (37), (38), (39), and (40) by their estimates obtained from Equations (34), (35), and (36).

VI. NUMERICAL EXAMPLE

Simply to illustrate the computational procedures for the bioassay estimates we will present in this chapter:

(a) Data for a hypothetical experiment in which 50 subjects exposed to each one of three dose levels are observed at the four successive times, 5, 10, 15, and 20 hours after the application of toxicant. These data have been calculated on the basis of postulated values of the transition intensities.

(b) Estimates of the parameters, ED_{50} and ET_{50}, obtained from the assumed transition intensities.

(c) A comparison of the estimates of ED_{50} and ET_{50} values in (b) with those obtained by applying the method of White and Graca (30).

A. Hypothetical Data

With four times of observations for each dose level there are, by section A, chapter V, fifteen mutually exclusive and exhaustive paths and, in Table 1, we present hypothetical data in which the numbers r_{AA}, r_{AMj}, r_{ADj}, and r_{MDjh} as defined in section A, chapter V, are indicated for each one of the fifteen paths.

As previously noted the data in Table 1 have been calculated from postulated values of transition intensities.

Table 1. Hypothetical data

Dose 1

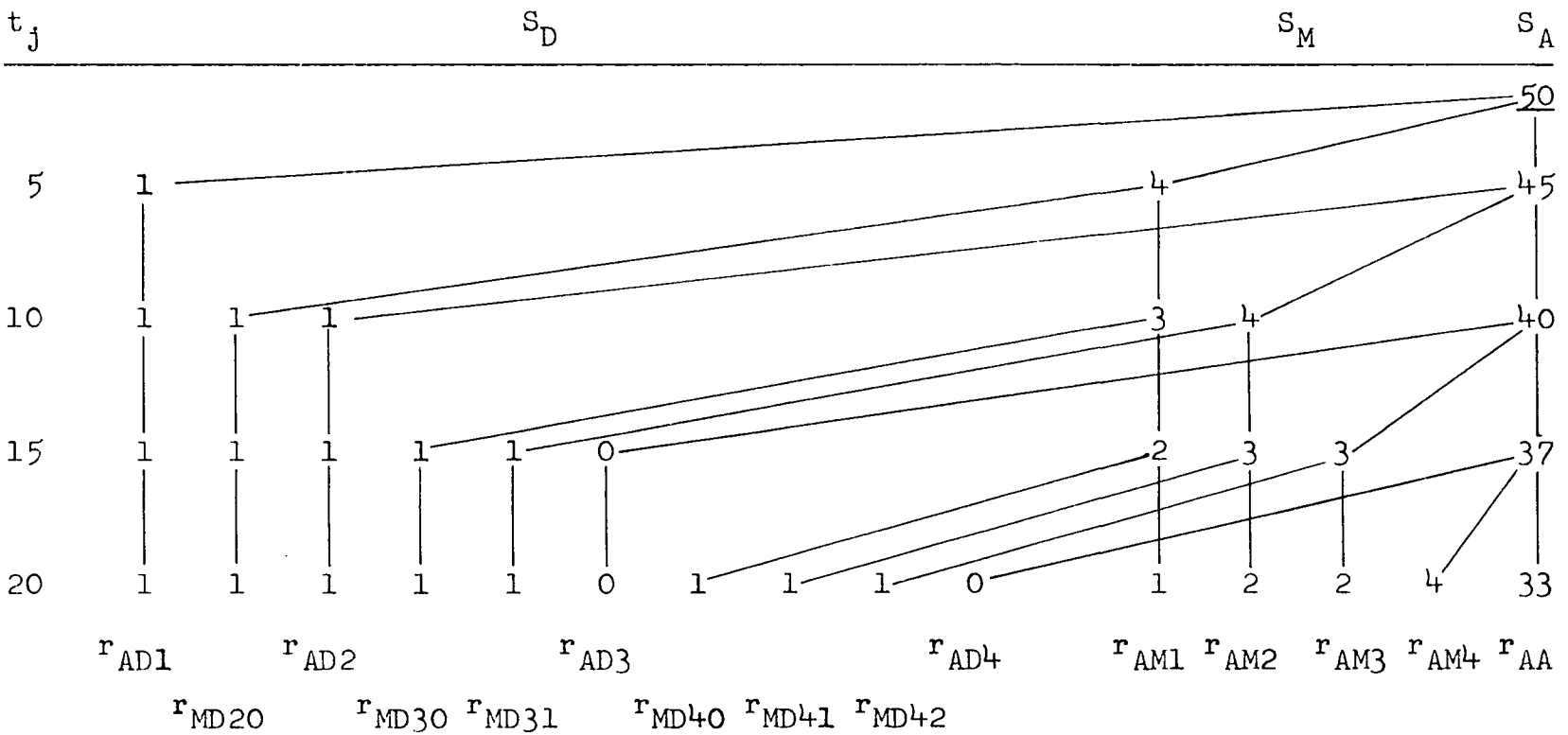


Table 1 (Continued).

Dose 2

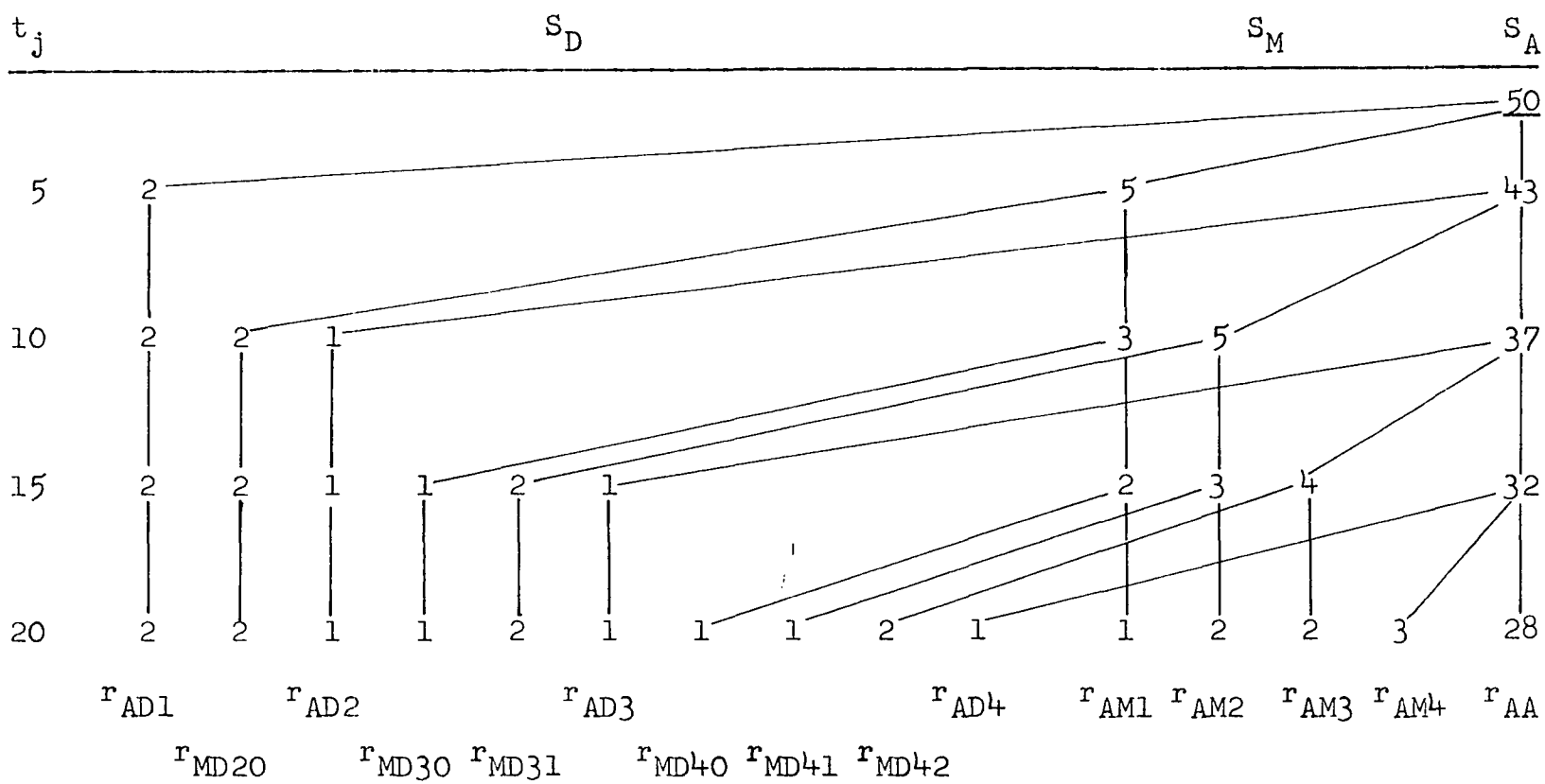
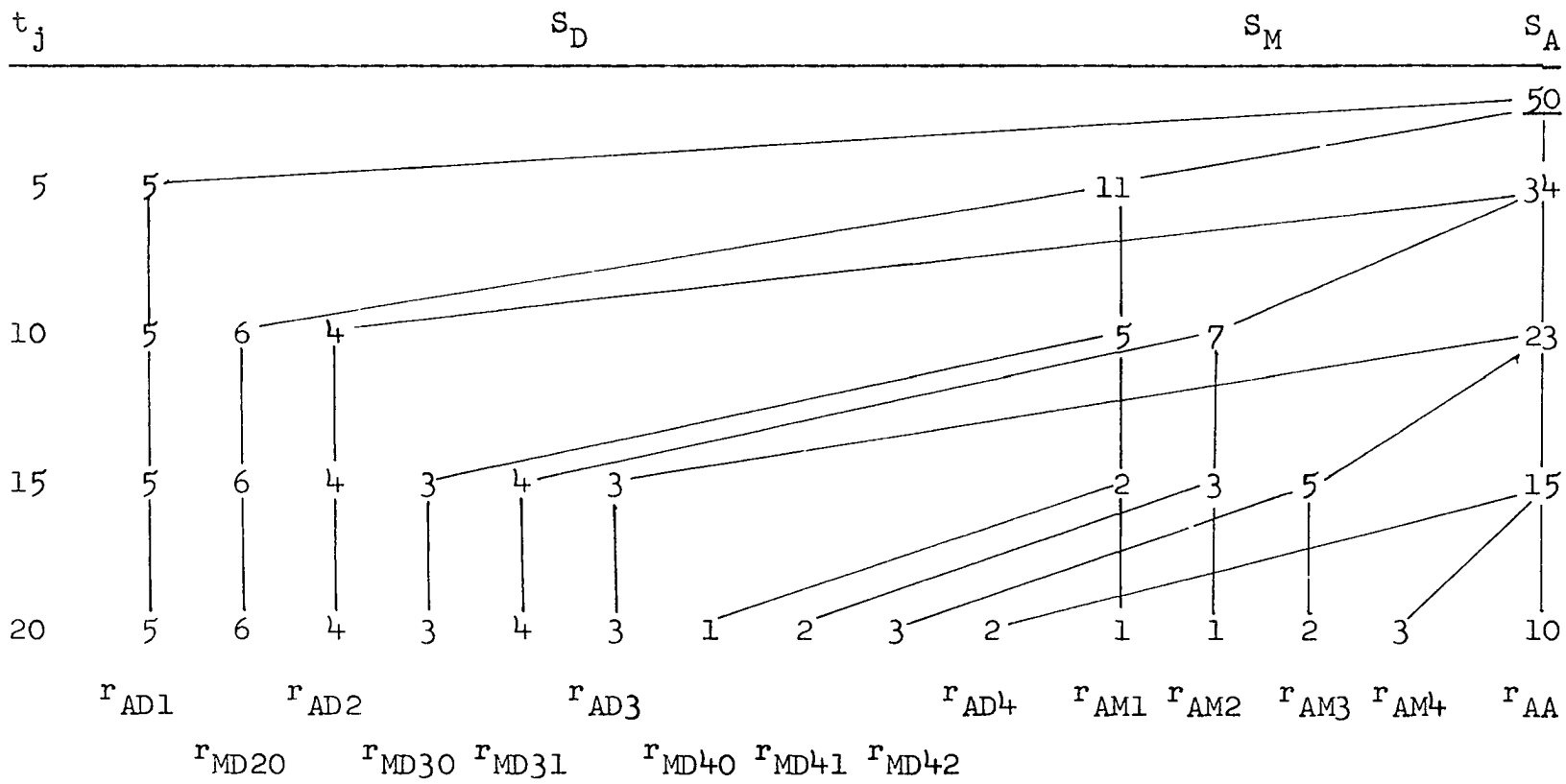


Table 1 (Continued).

Dose 3



These assumed intensities are indicated in Table 2 below for each dose level.

Table 2. The postulated transition intensities

Dosage	1.0	1.3	1.6
ξ_{AM}	0.02	0.05	0.08
ξ_{MD}	0.06	0.10	0.17

It would, of course, have been possible to obtain estimates of the transition intensities from the hypothetical data in Table 1 using the method described in chapter V. Whilst the required procedure is straightforward, the calculations are time consuming, particularly if only a desk machine is available. Accordingly the postulated transition intensities have been used in what follows.

Table 3 accordingly shows numerical values of the transition probabilities computed from the information given in Table 2 using the expressions in (10).

Table 3. The transition probabilities

Dose level	1	2	3
$P_{AA}(\tau)$	0.9048	0.8607	0.6703
$P_{AM}(\tau)$	0.0820	0.1089	0.2159
$P_{AD}(\tau)$	0.0132	0.0304	0.1138

Table 3 (Continued).

Dose level	1	2	3
$P_{MM}(\tau)$	0.7408	0.6065	0.4274
$P_{MD}(\tau)$	0.2592	0.3935	0.5726

It should be noted in Table 3 above that $\tau = 5$ hours for all probabilities in this particular case.

B. Estimates of the Parameters

1. The estimates of α, β, γ, and δ

The maximum likelihood estimates of the parameters α, β, γ, and δ obtained by the iterative procedure using the Equations 34, 35, and 36 are as follows:

$$\hat{\alpha} = -6.388, \quad \hat{\beta} = 2.371, \quad \hat{\gamma} = -4.706, \quad \hat{\delta} = 1.817$$

The estimates of the parameters obtained by the least squares method were used as trial values for the first cycle of iteration (see Appendix II for the justification of this procedure). Small residual values were found at the end of the first cycle and the corresponding solutions have been used for present purposes. Their accuracy could, of course, be improved by further iteration in any practical case.

2. The estimates of $ED_{50}(D)$

The $\underline{x}$ solution of the equation in (39) for the estimate of the $\hat{x}_{50}(D)$ dosage, with substitutions of $\hat{\alpha}$, $\hat{\beta}$, $\hat{\gamma}$, and $\hat{\xi}$ above, is also obtained by iteration. The estimate thus obtained is:

$$\hat{x}_{50}(D) = 1.50$$

3. The estimates of ED_{50} (D+M)

Applying the Equation 30:

$$\hat{x}_{50}(D+M) = \frac{\log_e(2^{\frac{1}{T}} - 1) - \hat{\alpha}}{\hat{\beta}}$$

with T = 20, $\hat{\alpha}$ = -6.388, $\hat{\beta}$ = 2.371 in this case, we obtained:

$$\hat{x}_{50}(D+M) = 1.34$$

C. Estimates of ED_{50} Based on Dichotomous Quantal Response

In Tables 4a and 4b are shown the 'equivalent' dichotomous quantal response data which have been obtained by combining the response classes 'moribund' and 'alive' given in Table 1.

Similarly Table 4b shows dichotomous quantal response data obtained when the response classes 'dead' and 'moribund'

are combined.

The estimates of $ED_{50}(D)$ and $ED_{50}(D+M)$ obtained from Tables 4a and 4b respectively, using the method of White and Graca will correspond to $\hat{x}_{50}(D)$ and $\hat{x}_{50}(D+M)$ obtained by the

Table 4a. Data obtained by combining the 'moribund' and 'alive' classes

	Dose 1		Dose 2		Dose 3	
t	D	M+A	D	M+A	D	M+A
5	1	49	2	48	5	45
10	3	47	5	45	15	35
15	5	45	9	41	25	25
20	8	42	14	36	33	17

Table 4b. Data obtained by combining the 'dead' and 'moribund' classes

	Dose 1		Dose 2		Dose 3	
t	D+M	A	D+M	A	D+M	A
5	5	45	7	43	16	34
10	10	40	13	37	27	23
15	13	37	18	32	35	15
20	16	34	22	28	40	10

present method. The pairs of corresponding estimates are shown in Table 5. It can be seen that corresponding values are in close, though not exact, agreement.

Table 5. Comparison of the ED_{50} estimates obtained by the two methods

	Present method	White and Graca method
$\hat{x}_{50}(D)$	1.50	1.46
$\hat{x}_{50}(D+M)$	1.34	1.33

In Table 6 are presented the estimates of $ET_{50}(D)$ and $ET_{50}(D+M)$ obtained by the present method and by the method of White and Graca for each one of the three pre-specified dosage levels. For application of the latter method a time metameter transformation is required. Examinations of the hypothetical data used have indicated that the logarithm of time could be very satisfactorily used for the time metameter.

Table 6. Comparison of ET_{50} estimates obtained by the two methods

	Present method		White and Graca method	
x	$\hat{t}_{50}(D)$	$\hat{t}_{50}(D+M)$	$\hat{t}_{50}(D)$	$\hat{t}_{50}(D+M)$
1.0	41.10	34.66	74.15	60.07
1.3	29.55	23.11	28.90	21.71
1.6	14.85	8.66	14.94	10.29

It can be seen from Table 6 that for the lowest dosage the present method gives estimates of ET_{50} substantially lower than those obtained by the method of White and Graca. The differences are small at the highest dosage level and it may be considered that these estimates are the more accurate since the dosage is then large enough to cover the point at which 50 per cent of the subjects were in fact affected. The question as to which one of the procedures is to be preferred will depend on the relevance of the two models to the underlying biological situation. This will be considered further in the chapter on discussion.

VII. DISCUSSION

A. Preliminary Remarks

It has been demonstrated in the foregoing chapters that it is possible to obtain estimates of the parameters in a practical bioassay based on the observation, for each subject, of quantal responses at each of a number of pre-specified times. Such estimates are, of course, subject to the validity of the biological and mathematical assumptions which have been made in the development. Some implications of these assumptions will now, therefore, be considered further.

B. Recapitulation of the Assumptions

1. Assumptions

The assumptions are that:

(a) the experimental subjects can be identified,

(b) the transfer from S_M to S_A does not occur at any time during the total period of the experiment,

(c) the transfer from S_A to S_D without the possibility of being observed in the state S_M does not occur in an infinitestimal time period,

(d) the total experimental time is relatively short so that the rates of moribundity and mortality due to causes other than the toxicant can be neglected,

(e) there exist conditional probabilities $P_{AA}(s,t)$, $P_{AM}(s,t)$, $P_{AD}(s,t)$, $P_{MM}(s,t)$, and $P_{MD}(s,t)$

which are continuous functions having partial derivatives at $t = s$,

(f) the biological situation can be represented by the mathematical model for a time homogeneous Markov process.

2. Discussions on the assumptions

(a) The assumption of identifiability will entail a restriction on the area of application of the method described, thus for example, it will not usually be possible to make such identifications in assays of insecticides using batches of insects. Other types of assays, for example, those in which small mammals are used as experimental subjects may, however, be amenable to the analysis described.

It should be noted, however, that, even when assumption (a) is not fulfilled, numbers of experimental subjects in two adjacent states may be combined to give a dichotomous classification which can be treated by the present procedure with a simple modification. In this dichotomous case the alternative analysis given by White and Graca is also available. The question as to which is the superior procedure will turn on the relevance of the alternative models to the practical situation.

This aspect has not here been given any general examination but a comparison of ED_{50} estimates obtained by the two methods in the numerical example has shown good agreement.

(b) Cases will certainly exist in which recovery from the state S_M to the state S_A is feasible so that the procedures presented in Chapters IV and V will not then be applicable. Such cases can, however, be analyzed using the methods outlined in Appendix I. On the other hand, it is also possible to envisage circumstances where assumption (b) is directly appropriate, as for example, when definite non-reversible physiological changes may occur in a subject following contact with some toxicant.

(c) It is not considered that this assumption is a strong restriction on the applicability of the procedures described, although some dramatic exceptions can be envisaged. The word 'moribund' has been used for convenience to distinguish the intermediate state but, in this connection, it is to be remembered that the analysis is applicable whenever there is at least one clearly recognizable state between the initial and final states.

(d) In many situations where the main objective of bioassay analysis is to estimate ED_{50}, the total experimental time is usually limited to a small number of hours. A short experimental time assists towards the validity of assumption (f) discussed below and is, therefore, desirable. In cases where the rates of natural and accidental mortality are not negligible, a preliminary study has shown that the present stochastic model can still be applied, with a simple modification, to estimate additional parameters.

(e) It is considered that the assumptions in (e) are reasonable ones for biological situations which are governed by the probabilistic laws. In particular the assumptions are generally accepted as the standard regularity conditions which define a birth process with continuous time parameter (9), (14).

(f) Assumption (f) is the basic assumption that the mathematical model used is relevant to the biological situation. The assumption has been made in numerous follow-up epidemiological studies in which individuals are invariably lost from observation and die from causes other than the one under study. In a number of such cases, the

assumptions may be clearly invalid, because the chances of changes in state from S_A to S_M or from S_A to S_D may increase or decrease during the duration of an experiment. For example, an individual subject, having spent some time in the state S_A may be developing some progressive resistance to further temporal exposure to the stimulus. On the other hand cases exist where the assumption is clearly a reasonable one particularly when the total experimental time is relatively short (and when the experimental conditions can be easily controlled). For example, when the same amount of a toxicant is administered to each one of the randomly selected individuals, the cumulative distribution of the individual tolerance times is approximately linear for some finite time interval over which the assumption is strictly valid.

In doubtful cases, the validity of the assumption can be tested from the results of preliminary experiments using a test (31) based on the ordinary χ^2 procedure.

3. Advantages and disadvantages of the present procedure

(a) the transition intensities for each dose level can be easily estimated as by-products of the

main analysis,

(b) the individual history on the response behavior of each subject is fully utilized to provide efficient estimators of the assay parameters,

(c) the principles of the present procedure can be applied to the cases where recovery occurs as outlined in Appendix I,

(d) only weak assumption on the time metameter is required.

The only assumption required on the time metameter is that, as given in (f) above, required for the Markov process to be time homogeneous. This is weaker than assumptions required by other procedures, for example, for the application of the White and Graca procedure a transformation has to be found empirically.

A disadvantage is that the iterative procedures required to estimate the parameters would be time-consuming, unless electronic computational facilities are available. This disadvantage is, of course, a common one in this situation.

Actual data to permit extensive investigations of the present approach have not so far been obtained. The results of applying this procedure to the hypothetical data are, however, very encouraging.

Finally it may be remembered that the procedures here described and, in particular, the introduction of the

Markovian principle, should be applicable to a wide variety of bioassay situations. It is hoped to give further examination to some of these cases.

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X. APPENDIX I. ESTIMATION OF ASSAY PARAMETERS WHEN THE ASSUMPTION OF NON-RECOVERY FROM THE MORIBUND STATE IS RELAXED

In this Appendix we will outline a procedure for estimating the parameters from a bioassay experiment in which recovery from S_M to S_A can occur in a finite time interval. In this case the transition probabilities, of which estimates are required, are:

$$P_{AA}^{(r)}(s,t),\quad P_{AM}^{(r)}(s,t),\quad P_{AD}^{(r)}(s,t),\quad P_{MA}^{(r)}(s,t),$$

$$P_{MM}^{(r)}(s,t),\quad P_{MD}^{(r)}(s,t) \tag{41}$$

where $P_{AM}^{(r)}(s,t)$, for example, is the probability that a subject observed in S_A at time s will leave S_A exactly r times, $r = 0, 1, \ldots$, in the time interval (t-s) and will be found in S_M at time t, $(t > s)$.

A. Assumptions

Our basic assumptions which specify this new model now become:

(a) the set of partial derivatives of the probability functions in (41) exists and may be written as follows:

(i) $\dfrac{\partial P_{AA}^{(0)}(s,t)}{\partial t} = \xi_{AA}$, $\dfrac{\partial P_{AM}^{(1)}(s,t)}{\partial t} = \xi_{AM}$,

(ii) $\dfrac{\partial P_{MA}^{(1)}(s,t)}{\partial t} = \xi_{MA}$, $\dfrac{\partial P_{MM}^{(0)}(s,t)}{\partial t} = \xi_{MM}$,

$$\frac{\partial P_{MD}^{(1)}(s,t)}{\partial t} = \xi_{MD}$$

all evaluated at t=s.

(iii) All other partial derivatives evaluated at t=s are zero.

(b) the probabilities given in (41) are continuous functions, with the following properties:

(i) $\lim_{t\to s} P_{AA}^{(0)}(s,t) = \lim_{t\to s} P_{MM}^{(0)}(s,t) = 1$,

(ii) $\lim_{t\to s} P_{AA}^{(r_1)}(s,t) = \lim_{t\to s} P_{MM}^{(r_5)}(s,t) = 0$,

(iii) $\lim_{t\to s} P_{AM}^{(r)}(s,t) = \lim_{t\to s} P_{AD}^{(r)}(s,t)$

$= \lim_{t\to s} P_{MA}^{(r)}(s,t) = \lim_{t\to s} P_{MD}^{(r)}(s,t) = 0$ (42)

B. The Probabilities as Functions of the Transition Intensities

With the assumptions given in section A above, the probability functions can be readily determined by applying the standard procedures described in, for example, (9), (11), and (14). As a brief illustration, we sketch the procedure which can be conveniently employed to obtain the probability functions $P_{AA}^{(r)}(s,t)$ and $P_{AM}^{(r)}(s,t)$.

The assumptions given in section A imply that the following system of differential equations exists:

$$\frac{\partial P_{AA}^{(r)}(s,t)}{\partial t} = \xi_{AA} P_{AA}^{(r)}(s,t) + \xi_{MA} P_{AM}^{(r)}(s,t),$$

$$\frac{\partial P_{AM}^{(r)}(s,t)}{\partial t} = \xi_{AM} P_{AA}^{(r)}(s,t) + \xi_{MM} P_{AM}^{(r)}(s,t) \qquad (43)$$

In order to solve the system in (43) we introduce the following generating functions with $0 \leq z \leq 1$:

$$G_{AA}(z,t) = \sum_{r=0}^{\infty} z^r \, P_{AA}^{(r)}(s,t),$$

$$G_{AM}(z,t) = \sum_{r=1}^{\infty} z^{r-1} \, P_{AM}^{(r)}(s,t) \qquad (44)$$

From (43) and (44) the following relations can be obtained:

$$\sum_{r=0}^{\infty} z^r \frac{\partial P_{AA}^{(r)}(s,t)}{\partial t} = \frac{\partial G_{AA}(z,t)}{\partial t} = \hat{\xi}_{AA} G_{AA}(z,t) + \hat{\xi}_{MA} z G_{AM}(z,t)$$

$$\sum_{r=1}^{\infty} z^{r-1} \frac{\partial P_{AM}^{(r)}(s,t)}{\partial t} = \frac{\partial G_{AM}(z,t)}{\partial t} = \hat{\xi}_{AM} G_{AA}(z,t) + \hat{\xi}_{MM} G_{AM}(z,t) \tag{45}$$

From the assumptions given in section A, it can now be seen that:

$$G_{AA}(z,s) = 1 \qquad \text{and} \qquad G_{AM}(z,s) = 0 \tag{46}$$

and, applying the usual methods for solution of the system (45) with the boundary condition in (46), we find:

$$G_{AA}(z,t) = \frac{\left\{f(z) + \hat{\xi}_{MM}\right\} e^{-f(z)(t-s)} - \left\{g(z) - \hat{\xi}_{MM}\right\} e^{-g(z)(t-s)}}{f(z) - g(z)}$$

$$G_{AM}(z,t) = \hat{\xi}_{AM} \frac{e^{-g(z)(t-s)} - e^{-f(z)(t-s)}}{f(z) - g(z)}$$

where

$$f(z) = -\tfrac{1}{2}\left(\hat{\xi}_{AA} + \hat{\xi}_{MM}\right) - \tfrac{1}{2}\left\{\left(\hat{\xi}_{AA} - \hat{\xi}_{MM}\right)^2 + 4z\,\hat{\xi}_{MA}\,\hat{\xi}_{AM}\right\}^{\frac{1}{2}}$$

and

$$g(z) = -\tfrac{1}{2}\left(\hat{\zeta}_{AA} + \hat{\zeta}_{MM}\right) + \tfrac{1}{2}\left\{\left(\hat{\zeta}_{AA} - \hat{\zeta}_{MM}\right)^2 + 4z\,\hat{\zeta}_{MA}\,\hat{\zeta}_{AM}\right\}^{\frac{1}{2}} \tag{47}$$

The estimation procedure in this general case would require a knowledge of the particular values r which, in turn, would necessitate continuous observation on each individual. This will not, in general, be practicable and we proceed to the case in which, as before, observations are made only at a number of pre-specified times.

C. Estimation of Parameters from Observations at Pre-specified Times

1. Biological assumptions

It is assumed in this case that any given interval between two pre-specified successive times s and t, $(t > s)$, is so short that:

(a) a subject observed as 'alive' at time s cannot during the interval (t-s) both become 'moribund' and recover at time t, and

(b) a subject observed as 'moribund' at time s cannot during the interval (t-s) both recover and become 'moribund' or 'dead' at time t.

2. Mathematical assumptions

In consequence of the preceding assumptions, the transition probabilities can now be written as follows:

$$P_{AA}(s,t),\quad P_{AM}(s,t),\quad P_{AD}(s,t),\quad P_{MA}(s,t),\quad P_{MM}(s,t),$$

$$P_{MD}(s,t)$$

where

$$\text{(a)}\quad P_{AA}(s,t) + P_{AM}(s,t) + P_{AD}(s,t) = 1$$

and

$$\text{(b)}\quad P_{MA}(s,t) + P_{MM}(s,t) + P_{MD}(s,t) = 1 \tag{48}$$

3. Transition probabilities

The transition probabilities can now be obtained by substituting $z = 1$ into (44). In fact, by inspecting (44), it can be seen that:

$$G_{AA}(1,t) = P_{AA}(s,t)$$

and

$$G_{AM}(1,t) = P_{AM}(s,t) \tag{49}$$

It immediately follows from (47) and (49) that these probability functions are:

$$P_{AA}(s,t) = \frac{(a + \hat{\xi}_{MM})\, e^{-a(t-s)} - (b - \hat{\xi}_{AA})\, e^{-b(t-s)}}{a - b}$$

and

$$P_{AM}(s,t) = \xi_{AM} \frac{e^{-b(t-s)} - e^{-a(t-s)}}{a - b}$$

where

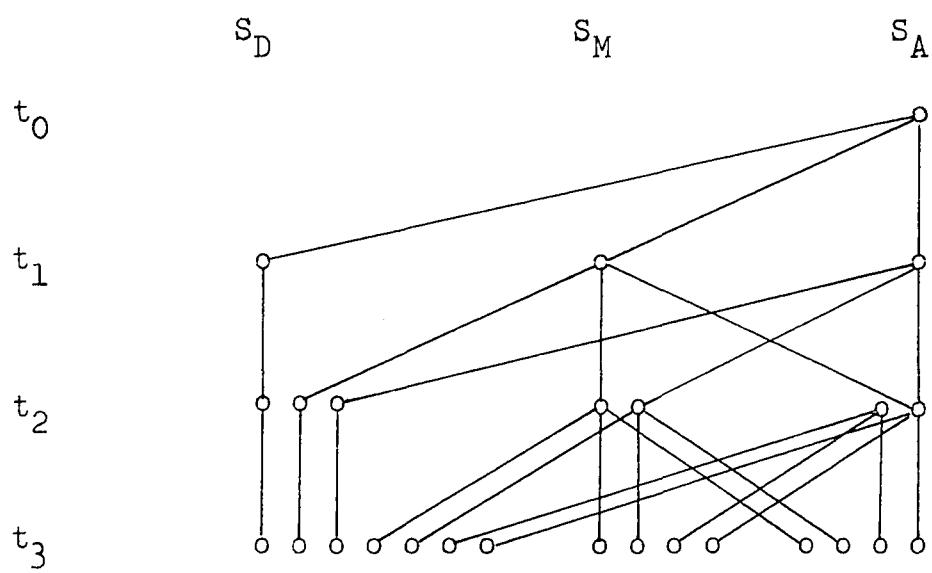
$$a = -\tfrac{1}{2}(\xi_{AA} + \xi_{MM}) - \tfrac{1}{2}\left\{(\xi_{AA} - \xi_{MM})^2 + 4\,\xi_{MA}\,\xi_{AM}\right\}^{\frac{1}{2}}$$

$$b = -\tfrac{1}{2}(\xi_{AA} + \xi_{MM}) + \tfrac{1}{2}\left\{(\xi_{AA} - \xi_{MM})^2 + 4\,\xi_{MA}\,\xi_{AM}\right\}^{\frac{1}{2}} \qquad (50)$$

The probability functions $P_{MA}(s,t)$, $P_{MM}(s,t)$, and $P_{MD}(s,t)$ can be found similarly.

4. Alternative paths

A scheme representing the three states and the passage of transfer is given in Figure 2 below for the first three successive time intervals.



(Figure 2)

Denote by Q_{Ajh}, Q_{Mjh}, Q_{Djh} the mutually exclusive classes as follows:

(a) Q_{Ajh}: the class of (2^{j-1}) paths through which an individual subject in S_M or S_A at t_{j-1} is transferred to S_A in $(t_j - t_{j-1})$,
$h = 1, \ldots, j-1$,
$j = 1, \ldots, k$

(b) Q_{Mjh}: the class of (2^{j-1}) paths through which an individual subject in S_M or S_A at t_{j-1} is transferred to S_M in $(t_j - t_{j-1})$,
$h = 1, \ldots, j-1$,
$j = 1, \ldots, k$

(c) Q_{Djh}: the class of $(1+2+2^2+, \ldots, +2^{j-1})$ paths through which a subject in S_A or S_M or S_D at t_{j-1} is transferred to S_D in $(t_j - t_{j-1})$,
$h = 1, \ldots, j-1$,
$j = 1, \ldots, k$

where the subscript $\underline{h}$ within any one of the classes Q_{Aj}, Q_{Mj}, Q_{Dj} distinguishes the history of a subject at time h, $h = 1, \ldots, j-1$. At the end of the final time interval k, the numbers of the mutually exclusive paths are therefore:

(a) (2^{k-1}) paths in class Q_{Akh}

(b) (2^{k-1}) paths in class Q_{Mkh}

(c) $(\sum_{u=0}^{k-1} 2^u)$ paths in class Q_{Dkh}

giving a total of

$$\left(\sum_{u=0}^{k} 2^u\right)$$

mutually exclusive possible alternative paths at time t_k.

5. Estimation of the transition intensities

Let r_{Ajh}, r_{Mjh}, and r_{Djh} denote the observed numbers corresponding to the paths Q_{Ajh}, Q_{Mjh}, Q_{Djh} respectively at the end of the experiment. Then the set of random variables r_{Ajh}, r_{Mjh}, and r_{Djh} is multinomially distributed. The likelihood function of the observations for a given dose level, which is similar to that given in (15), is:

$$e^L = C' \cdot \prod_j \prod_h \left[\Pr\{Q_{Ajh}\}\right]^{r_{Ajh}} \left[\Pr\{Q_{Mjh}\}\right]^{r_{Mjh}} \left[\Pr\{Q_{Djh}\}\right]^{r_{Djh}} \tag{51}$$

where C' is a constant and $\Pr Q_{Ajh}$ etc. are the probabilities corresponding to the path Q_{Ajh} etc., which can be obtained from the expressions in (50).

It should be noted, however, that there are now three indipendent transition intensities ξ_{AM}, ξ_{MA}, and ξ_{MD} to be estimated. The estimating equations are obtained by differentiating the logarithm of the likelihood function given in (50).

As in the case of non-recovery, explicit solutions for the intensities do not exist and, therefore, iterative procedures will also be required here to estimate the intensities.

6. Estimation of the assay parameters

The direct extension of the principles described in chapter V can be applied to the present case for estimating the parameters. Let the relation between the new intensity $\hat{\xi}_{MA}$ and a suitable transformation (such as the logistic function) be represented by:

$$\hat{\xi}_{MA} = g(\nu, \eta, x) \tag{52}$$

where ν and η are unknown parameters and x is, as usual, the dosage. Then the likelihood function for the intensities will be of the form:

$$L = h(\alpha, \beta, \gamma, \delta, \nu, \eta, x, t) \tag{53}$$

where α, β, γ, δ, ν, and η are the parameters as defined in (25), (27) and (52). These parameters can be estimated by the iterative procedures, as before, using the estimating equations which may be obtained by differentiating (53).

The $ED_{50}(D)$ and $ET_{50}(D)$ are then found as the dose and time respectively for which:

$$\sum_j \sum_h \Pr\left\{Q_{Djh}\right\} = \tfrac{1}{2} \tag{54}$$

The $ED_{50}(D+M)$ and $ET_{50}(D+M)$ will be similarly obtained from the relation:

$$\sum_{j}\sum_{h}\left[\Pr\left\{Q_{Mjh}\right\} + \Pr\left\{Q_{Djh}\right\}\right] = \tfrac{1}{2} \qquad (55)$$

XI. APPENDIX II. LEAST SQUARES PROCEDURE FOR OBTAINING FIRST TRIAL VALUES OF THE ASSAY PARAMETERS

Here, we will outline a procedure for obtaining a set of trial values which may be used for the first cycle of iteration in Equations (34), (35), and (36). From the relations given in (25) and (27) we obtain, by taking logarithms:

$$\log\left(e^{\xi_{AMi}} - 1\right) = \alpha + \beta x_i$$

and

$$\log\left(e^{\xi_{MDi}} - 1\right) = \gamma + \delta x_i \qquad i=1, \ldots, m \qquad (56)$$

Suppose now that the intensities ξ_{AMi} and ξ_{MDi} have been estimated by using the estimating equations in (18) and (19). Denote these estimates by

$$\hat{\xi}_{AMi} \quad \text{and} \quad \hat{\xi}_{MDi}$$

respectively and consider the following models:

$$\log\left(e^{\hat{\xi}_{AMi}} - 1\right) = \alpha + \beta x_i + e_i$$

$$\log\left(e^{\hat{\xi}_{MDi}} - 1\right) = \gamma + \delta x_i + f_i \qquad i=1, \ldots, m \qquad (57)$$

where both e_i and f_i are assumed to be the random errors which satisfy the usual Gauss-Markov conditions. Then the

least squares estimators of α and β are:

$$\hat{\beta} = \frac{1}{\sum_i (x_i - \bar{x})^2} \sum_i (x_i - \bar{x})^2 \log (e^{\hat{\xi}_{AMi}} - 1)$$

$$\hat{\alpha} = \frac{1}{m} \sum_i \log (e^{\hat{\xi}_{AMi}} - 1) - \hat{\beta}\bar{x} \qquad (58)$$

Similar expression can be used for the least squares estimators of γ and δ and it may be noted that these estimates can be very quickly obtained.